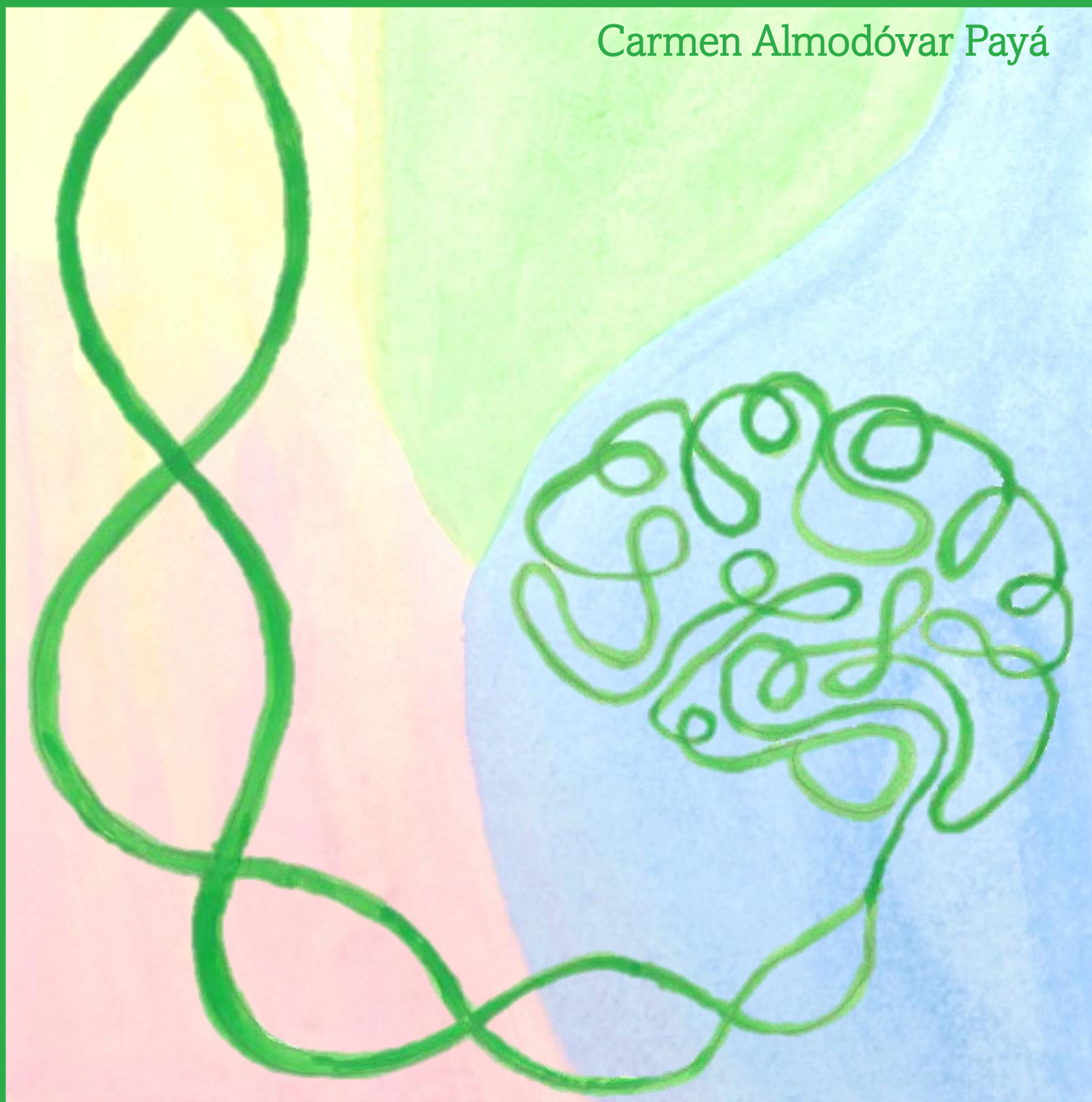
UNIVERSITAT DE BARCELONA

The role of the synaptic plasticity gene NRN1 in schizophrenia: integrating molecular and neuroimaging approaches

Carmen Almodóvar Payá

The role of the synaptic plasticity gene *NRN1* in schizophrenia: integrating molecular and neuroimaging approaches

Carmen Almodóvar Payá



Doctoral Thesis • **Carmen Almodóvar Payá** • 2024

FIDMAG Germanes Hospitalàries Research Foundation

Department of Evolutionary Biology, Ecology and Environmental Sciences

Doctoral Program in Biomedicine

The role of the synaptic plasticity gene *NRN1* in schizophrenia: integrating molecular and neuroimaging approaches

El paper del gen de plasticitat sinàptica NRN1 en l'esquizofrènia: integració de dades moleculars i de neuroimatge

Thesis submitted by:

Carmen Almodóvar Payá

to qualify for:

Doctorate degree by the Universitat de Barcelona

Doctorate candidate

Carmen Almodóvar Payá

Director

Mar Fatjó-Vilas Mestre

Co-director and tutor

Bárbara Arias Sampériz

Funding sources

This predoctoral research has been conducted thanks to the following funding sources:

Project grants and research entities:

- Research project by Fundación Alicia Koplowitz.
- Research project by Acadèmia de les Ciències Mèdiques i de la Salut de Catalunya i Balears.
- Research projects PI18/1535 and PI20/01002, Instituto de Salud Carlos III. Spanish Ministry of Science and Innovation. Co-founded by European Regional Development Fund "A way to make Europe" and European Social Fund "Investing in your future".
- Research project 41/C/2022 by Fundació La Marató de TV3, Barcelona, Spain.
- Grant (ID 26206) by NARSAD Young Investigator Research Award from the Brain and Behaviour Research Foundation.
- Centro de Investigación Biomédica en Red de Salud Mental – CIBERSAM.
- Consolidated Research Group 2021SGR1475, Comissionat per a Universitat i Recerca del DIUE (Agència de Gestió d'Ajuts Universitaris i de Recerca – AGAUR)

Als meus iaios, pel vostre amor, generositat i bondat.

A tu, iaio, per portar sempre un xiulit i un somriure com a bandera, per despertar-me cada matí cantant-me Caperucita, i per regalar-me la infància més feliç entre bancals i tomateres. A tu, iaia, pel teu voler tan bonic i la teua sensibilitat; el record més preciós que guarde és anar caminant amb tu de la maneta. A tu, teta, per la teua valentia i alegria contagioses, per totes les teues passaetes, i pel teu llenguatge inventat, sense el qual ja no puc entendre la vida.

No hi ha dia que no pense en vosatros.

Agraïments

Sempre m'ha generat un poc d'ansietat escriure la secció d'agraïments de la tesi, ja que no sóc especialment bona expressant les meues emocions. No obstant això, reflexionar sobre estos anys m'ha impressionat i m'ha fet adonar-me del privilegi que és haver-me creuat pel camí amb persones com vosaltres. La burocràcia que fa que este procés culmine amb una escriptura i defensa individual de la tesi em sembla injusta perquè no reflecteix tot el treball i l'esforç col·lectiu que hi ha darrere. Sé que esta experiència no és universal i que moltes persones passen per esta etapa en solitud. Però no és el meu cas. Sempre he comptat amb persones que m'han ajudat a imaginar preguntes, a formular-les adequadament, a respondre-les de manera creativa, a revisar textos, a unir les peces per explicar la història de manera més clara, a proporcionar referències, a gestionar submissions, a condensar idees en pòsters, a recordar *deadlines*, a comunicar idees, a alinear imatges, a clicar conjuntament el botó de *send* de milers de beques, a abraçar-me quan alguna cosa em sobrepassava, a fer-me riure quan estava trista, i fins i tot a cuinar-me i a pentinar-me. Persones que han dedicat molts esforços a tasques que poden parèixer xicotetes, però que són realment importants. Tot ha sigut un esforç conjunt i de suport mutu. Per això, estic encara més agraïda i impressionada de tenir-vos a totes i espere poder fer-vos veure el que realment sou: genials. O com diria el meu gran referent M. Rajoy: *"Da gusto hablar bien de quien se merece que hablen bien de uno, y de aquel del que uno quiere hablar bien porque se lo merece"*.

No puc començar estos agraïments amb una altra persona que no sigues tu, Mar. Em vas agafar en aquella adolescència eterna dels 27 anys, o millor dit, una crisi avançada dels 30. Em vas mostrar que la recerca no és el que ens ensenyen a les classes de biomedicina, que hi ha xicotetes repúbliques on la competència no mana, on preval el companyerisme, i on el que realment importa és disfrutar del camí. Gràcies per confiar en mi, però sobretot, per valorar-me i escoltar els meus punts de vista, per motivar-me, per fer que el currículum siga tan llarg com un dia sense pa, per apretar-me quan ho necessitava però també per donar-me espai, per ensenyar-me que no hi ha una única resposta, sinó que tot depén de la pregunta que et faces, per ajudar-me a superar el meu temor a la pàgina en blanc ("Carmen, escriu, escriu, escriu!"), per ensenyar-me a trobar el fil i a contar la història, però sobretot, per protegir-me de les traves diàries que fan que la recerca, de vegades, parega un laberint d'obstacles absurd. I com no, gràcies per portar-nos a tots els congressos, per les rialles amb neveres que fan d'armaris, amb sopars amb llagostes per picar, amb senyores jubilades atrapades dins d'un avió, i una infinitat d'anècdotes que hem viscut i que fan que recorde este període amb molt d'afecte. Però, més enllà dels aspectes laborals, vull agrair-te com m'has cuidat personalment. Les vegades que m'has telefonat quan he acabat a urgències per al·lèrgies o infeccions, les cervesetes, els trivials als bars, els dinars per celebrar qualsevol èxit, o les converses al teu cotxe. Em sent molt agraïda d'haver-te tingut, no només com a directora de tesi, sinó com a companya durant tots estos anys.

A l'altre pilar d'aquesta tesi, Bárbara, tot i que sempre et lleves mèrits, saps que estic immensament agraïda d'haver-te tingut com a codirectora. No només per aguantar-me cada vegada que m'apareixia al despatx, per fer broma de tot i per riure't de mi les cinquanta mil vegades que estes últimes setmanes he dit "estic bé, estic bé, estic bé!", sinó per estar sempre al meu costat. M'has permès demanar beques amb tu, posar mostres a les teues plaques i, per si fora poc, vas confiar en mi per fer les teues classes, obrint-me així la porta de la docència. Però, sobretot, t'agraïsc que m'hages tractat sempre amb un afecte immens, amb bones paraules per a tot, des dels articles fins a les revisions i tot el que hi ha pel mig. Espere que sàpigues que això ha sigut una font de motivació i seguretat enorme per a mi.

També vull agrair a l'Edith, directora del grup, per haver-me obert les portes de FIDMAG donant-me l'oportunitat de formar part d'aquest equip tan compromès i de tanta qualitat.

Un agraïment enorme també a tot NeuroBioGen, Noemí, Jan i Mariona, però també als infiltrats del grup, Irene i Dani, perquè heu convertit les hores de treball en moments increïblement divertits, des de les mil hores compartides dinant i arreglant el món, fins a les cerveses i els jocs de taula, els *scape rooms*, els trivials, i alguna que altra festa. No puc no fer-li una menció especial a la meua Marioni, perquè eres la meua aquari preferida. És que no podia ser que no fórem del mateix dia! Ja saps que pense que eres la persona més llesta del món, però a part d'això, personalment també eres increïble. No saps quantes coses he aprés de tu. Espere que siguem amiguetes sempre!

I una altra menció especial a la meua valencianeta preferida, Maria, perquè ningú sap la sort que és haver-te tingut com a companya de doctorat. No saps com d'estrany va ser deixar de tindre't al meu costat, literalment cadira amb cadira durant 12 hores al dia. Tot i així, tot el que hem compartit, almenys en el terreny laboral, queda plasmat en algunes de les imatges d'aquesta tesi que no són meues, sinó teues. Vaig pensar en canviar-les, però em semblava bonic que algú les mirara anys més tard i pensara "mira estes, que van fer dos tesis entre les dos", perquè així ha sigut. Fins i tot ens vam traure un C2 d'anglés juntes! I en el terreny personal, què dir-te, són ja tants anys i tantes coses compartides que no cabrien aquí. És un gust saber que sempre ens tindrem l'una a l'altra. I aprofite per agrair a la meua Normi, la italiana más intensa y *punk* a la vez… ¿cómo se explica eso? Ojalá pronto volvamos a hacer un viaje juntas. Os adoro!

Gràcies també a tot l'equip de FIDMAG. Gràcies a Pilar, aquell treball de final de màster que em vas codirigir ja queda molt lluny, però sempre t'agrairé haver-me introduït en el món de la neuroimatge i per estar sempre disposada a ajudar-me quan ho necessite, ja siga amb els scripts de FreeSurfer, de Matlab o amb les visualitzacions dels clústers. També voldria agrair al personal d'infermeria CASM de Benito Menni que sempre ens ha ajudat en la recollida de mostres.

La gent del Departament de la UB també ha format part d'esta tesi. Gràcies a Lourdes, Esther, Neus, Laura i Yorgos. Vull fer una menció especial a l'Araceli, per tota l'ajuda que m'has oferit. Gràcies per intentar que els contractes sempre s'allargaren un poc més, per confiar en mi per fer moltíssimes hores de docència i ajudar-me a quadrar-les totes, però alhora, per ser sempre comprensiva i procurar posar el doctorat per davant. Gràcies també per les teues bones paraules i per l'ànim que m'has donat constantment. Quiero agradecer también a los predocs: Marina, Miriam, los Luises, las Nereas y Patricia, por acogerme siempre en vuestras comidas, por el *pressing* para ir a la BioFesta y por los Aperols y las focaccias florentinas.

També vull agrair a Carme Gallego i Marcos Moreno, perquè una part fonamental d'esta tesi ha eixit endavant gràcies a vosaltres. Em fa molt feliç saber que esta tesi no siga un punt i final i que encara tinguem projectes conjunts per davant, Carme. I a tots els investigadors, tan basics com clínics, dels projectes i dels grups col·laboradors del CIBERSAM sense els quals este treball no seria possible.

Part of this thesis also reflects the time I spent at the Experimental and Clinical Research Center (ECRC), Charité – Universitätsmedizin, in Berlin. I extend my sincere gratitude to Professor Chotima Böttcher for the opportunity to join her group and for introducing me to the field of CyTOF. It was a privilege to work alongside her team. Però, si em porte alguna cosa d'esta estada, és haver complit el somni de treballar amb tu, Cami. Qui ens anava a dir que faríem un doctorat i, amb sort, un article juntes! Eres la persona més extraordinària i divertida que conec, l'amiga que tot el mon voldria tenir, i em sent molt afortunada. Hem disfrutat moltíssim a Barcelona, però també a Brussel·les i Berlín, perquè saps que, allà on vages, jo sempre vindré a fer-te la visita de rigor. Perquè ojalà visquérem a la mateixa ciutat!

També m'agradaria agrair als meus amics de tota la vida. Gracias a mis alicantinas favoritas, Isa, Nadia y Rocío. Porque, aunque nunca entendisteis del todo mi manía de venirme a vivir a Barcelona, siempre me habéis apoyado sin condiciones. Porque cada vez que estamos juntas, siento que no ha pasado el tiempo. També a totes les amigues de Petrer, Natalia, Maria, Sílvia, Reme, Estefanía, i especialment, a la meua Mariatillo, ojalà puguem anar a un festival juntes als EEUU per celebrar la tesi! Et trobe molt a faltar. I als petrerins per Barcelona, Carlos i Carles, gràcies, perquè també m'heu aguantat en esta recta final, vos mereixeu un arrosito. Gràcies a les meues eivissenques, per no permetre que em tanque en la meua bombolla i arrossegar-me sempre a viatges on el destí important ben poc. Sou genials totes!

Per suposat, vull dedicar un agraïment molt especial a la meua família. Sobretot als meus pares i a ma tia. Quina sort tenir una família que et recolza quan caus, que celebra cada èxit com si fora propi i que expressa el seu amor obertament. Pense en la iaia i la teta quan deien "nena, tu tens que ser valenta!", i crec que de vegades ho sóc, però clar, és que tinc la certesa de comptar amb una xarxa de suport enorme disposada a fer el que siga per mi. Gracias, papá, por transmitirme tu radicalidad y por enseñarme a ver la

vida sin miedo a cuestionarlo todo. Has sido la persona que más me ha escuchado, quien ha estado a mi lado siempre y quien mejor me conoce. Gracias por esa sensibilidad especial y por todo lo que has compartido conmigo. Gràcies, mama, per la confiança incondicional que sempre has tingut en mi, per creure que puc amb tot, fins i tot quan jo mateixa tinc dubtes. Gràcies per estar tan orgullosa de mi, per celebrar cada èxit com si fora teu i per recordar-me cada dia que l'esforç val la pena. Gràcies, tia, per les teues paraules d'amor incondicional, per estar sempre pendent de mi i per no deixar que em descuide ni em deixe arrossegar pel ritme frenètic de la vida. Gràcies per enviar-me sempre la teua energia positiva, que em fa sentir-te prop encara que estigues lluny. Gràcies per ser eixe pilar constant que em dona força, ja siga per afrontar grans decisions o per trobar la confiança per entrar a un *hostel* en Londres. Vos vull moltíssim!

Particularment, vull agrair a la meua germana xicoteta, Aitaneta. No sé ni per on començar. De ben xicotetes, i a pesar de la diferència d'edat, sempre ho hem passat genial juntes. Soc feliç de saber que sempre vols formar part de tot el que em passa, ja siga venint a visitar-me a tots els països pels quals he passat o fent-me una portada preciosa per a la tesi. Però sobretot, m'encanta que me deixes dormir amb tu sempre, que tingues eixes manetes tan gustosetes, les nostres rutines cinematogràfiques de nadal, i en general, saber que sempre estàs ahí quan te necessite, cuidant-me i pujant-me la moral. Te vull "hasta el infinito"!

I, molt especialment, vull agrair a Cristian, ja saps que eres la meua persona favorita del món per moltes raons, però sobretot perquè tens la virtut de fer que el millor dia siga aquell que passem tirats al sofà sense fer res. M'encanta que ens retroalimentem en gustos musicals, llibres, idees, menjars... eixa bombolla increïble de la qual mai voldria eixir. Gràcies per ser un suport constant. Te vull moltíssim!

I, com no, també vull agrair a tota la resta de la família: a mon tio Paco, als meus germans, Paula, Borja i Enrique, al baloncestista de la família, Dariet, als cosins Francisco i Ana, a la més llesta i guapa de totes, Lola, i als més xicotets, Mario, Marc i Pablo. I no m'oblide dels meus cunyaets, Javi, Miriam, Davidet i Alba. I mon tio Mario, la persona més propera a les tres que més he volgut mai i qui, com elles, millor pot entendre el que significa haver arribat fins aquí. De veritat, ojalà tots tinguérem la teua vitalitat!

I per suposat a totes les persones que decidiu participar en estudis d'investigació, sense la vostra generosa col·laboració esta tesi no hauria estat possible.

Gràcies a totes!

Abstract

Schizophrenia, a psychiatric disorder affecting 24 million people worldwide, continues to present significant challenges despite notable progress in understanding its etiology. While advancements have been made, the fundamental pathophysiological mechanisms, molecular diagnostics, and precise biomarkers remain unclear. Consequently, ongoing psychiatric research must focus on unraveling the complex biological foundations of the disorder. A deeper understanding is crucial for identifying more appropriate diagnostic categories and developing innovative therapeutic strategies to improve the quality of life for individuals affected by schizophrenia.

Currently, the leading etiological hypothesis suggests that schizophrenia arises from a complex interaction between genetic and environmental factors, which disrupt the processes governing brain development and maturation. From birth to adulthood, brain development depends on synaptic plasticity, the process by which neurons modify their connections in response to external signals, refining synapses and creating neural circuits. Accordingly, impaired synaptic plasticity is thought to play a pivotal role in the pathophysiology of schizophrenia, a notion strongly supported by evidence from multiple disciplines.

Among the genes that play crucial roles in both development and adulthood by mediating synaptic plasticity, Neuritin-1 (*NRN1*) stands out. Prior to this thesis, extensive research using cell and animal models had explored the functions of *NRN1* in the brain. In summary, *NRN1* regulates apoptosis in proliferative neurons, promotes neuronal migration and synaptic maturation, modulates neurite outgrowth during differentiation, stimulates dendritic arbor growth, stabilizes active synapses, and as previously mentioned, supports synaptic plasticity. Additionally, several studies have highlighted *NRN1's* neuroprotective effects, its role in enhancing cognitive performance, and its sensitivity to neurotherapeutic agents through epigenetic mechanisms. This body of evidence suggests that *NRN1* could be a promising target for developing therapeutic strategies for schizophrenia, as modulating its expression may positively impact brain functioning and, therefore, behavior and patient outcomes. However, despite these promising findings, human research on the role of *NRN1* in schizophrenia remained limited, with only two previous studies examining its impact on schizophrenia risk, age at onset, and intelligence quotient.

Given its potential importance, we focused on *NRN1* as a key synaptic plasticity gene in schizophrenia, aiming to deepen our understanding of how this mechanism contributes to the disorder and how such knowledge could enhance patient care. To achieve this, we adopted a multilevel approach incorporating various layers of *NRN1* molecular diversity. This strategy resulted in four articles that examined the association of *NRN1* with age at onset, clinical and neuroimaging traits of schizophrenia, its interactions with related

genes, its expression and methylation in post-mortem brain samples from schizophrenia patients, and its methylation changes in response to cognitive therapy.

Overall, our results validate the efficacy of a multifaceted methodology in uncovering the role of candidate genes in modulating schizophrenia presentation. Specifically, we identified *NRN1* genetic variants associated with an increased risk for early-onset schizophrenia-spectrum disorders, with carriers exhibiting altered dorsolateral prefrontal cortex activity during a working memory task. Additionally, epistatic interactions between *NRN1*, *BDNF*-rs6265, and *CACNA1C*-rs1006737 significantly modulated clinical severity and neuroanatomical features, with the interaction between *NRN1* and *BDNF* in the left lateral orbitofrontal cortex fully mediating the effects on general psychopathology. In post-mortem brain samples, schizophrenia patients treated with clozapine displayed lower *NRN1* expression and methylation differences in the prefrontal cortex compared to untreated patients and controls, with these methylation differences correlating with *NRN1* expression and influenced by specific genetic variants. Lastly, we demonstrated the clinical applicability of our findings, showing that cognitive remediation therapy is associated with changes in *NRN1* peripheral methylation, with increased methylation distinguishing responders from non-responders across cognitive domains, an effect also shaped by *NRN1* genetic variability.

Globally, the findings presented in this thesis highlight how understanding the role of specific genes involved in synaptic plasticity can not only deepen our insight into the etiology of schizophrenia but also potentially lead to improved patient care. Although studies focusing on candidate genes are constrained by the polygenic nature of the disorder, these studies provide compelling evidence and valuable clinical insights, laying the groundwork for further exploration of these key genes within broader molecular networks. Thus, this thesis serves as a steppingstone in the collective process of building knowledge about the etiology of psychosis, aiming to understand how individual genetic variations influencing neurodevelopment and synaptic plasticity contribute to the underlying causes of schizophrenia.

Keywords

Psychotic disorders, synaptic plasticity, genetics, methylation, *NRN1*

Resum

L'esquizofrènia, un trastorn psiquiàtric que afecta 24 milions de persones arreu del món, encara presenta grans reptes pel que fa a la comprensió dels seus mecanismes etiològics. Malgrat els avenços en aquest camp, els mecanismes bàsics que la causen, així com el seu diagnòstic molecular i l'ús de biomarcadors precisos, continuen sent poc clars. Per tant, la investigació psiquiàtrica ha de centrar-se a desxifrar els fonaments biològics complexos del trastorn. Això és fonamental per definir millor les categories diagnòstiques i crear noves estratègies terapèutiques que millorin la qualitat de vida de les persones amb esquizofrènia.

La principal hipòtesi etiològica suggereix que l'esquizofrènia és el resultat d'una interacció complexa entre factors genètics i ambientals que altera els processos que guien el desenvolupament i la maduració del cervell. Al llarg del desenvolupament, des del naixement fins a l'edat adulta, el cervell depèn de la plasticitat sinàptica, un procés en què les neurones adapten les seves connexions en resposta a estímuls externs, refinant les sinapsis i formant circuits neuronals. Així, es creu que les alteracions en la plasticitat sinàptica són centrals en la fisiopatologia de l'esquizofrènia, una idea fermament recolzada per evidència de diverses disciplines.

Entre els gens que tenen un paper crucial en el cervell, tant en el desenvolupament com en l'edat adulta contribuint a la plasticitat sinàptica, destaca especialment Neuritin-1 (*NRN1*). Diversos estudis amb models cel·lulars i animals previs a aquesta tesi ja havien explorat les funcions de *NRN1* en el cervell. En resum, *NRN1* regula l'apoptosi en neurones proliferatives, promou la migració neuronal i la maduració sinàptica, modula el creixement de neurites durant la diferenciació neuronal, estimula el desenvolupament de les dendrites, estabilitza les sinapsis actives i, com ja s'ha esmentat, dona suport a la plasticitat sinàptica. A més, diversos estudis han subratllat els efectes neuroprotectors de *NRN1*, el seu paper en la millora del rendiment cognitiu i la seva sensibilitat als agents neuroterapèutics mitjançant mecanismes epigenètics. Aquest conjunt d'evidències suggereix que *NRN1* podria ser un gen prometedor per al desenvolupament d'estratègies terapèutiques per a l'esquizofrènia, ja que modular la seva expressió podria impactar positivament en el funcionament cerebral i, per tant, en el fenotip dels pacients. Tot i aquestes troballes prometedores, la recerca en humans sobre el paper de *NRN1* en l'esquizofrènia era limitada, amb només dos estudis previs que examinaven el seu impacte en el risc d'esquizofrènia, l'edat d'aparició del trastorn i el quocient intel·lectual dels pacients.

Atès el seu gran potencial, ens vam centrar en *NRN1* com a gen essencial en la plasticitat sinàptica en l'esquizofrènia, amb l'objectiu d'aprofundir en la comprensió de com aquest mecanisme contribueix al trastorn i com aquest coneixement podria millorar l'atenció als pacients. Per aconseguir-ho, vam adoptar un enfocament de múltiples nivells que integra diverses capes d'anàlisi de la diversitat molecular de *NRN1*. Aquesta estratègia va resultar en quatre articles que examinen l'associació de *NRN1* amb l'edat

d'inici de la malaltia, diferents trets clínics i de neuroimatge, les seves interaccions amb gens relacionats, la seva expressió i metilació en mostres cerebrals post-mortem de pacients amb esquizofrènia, i els canvis de metilació en resposta a la teràpia cognitiva en aquests pacients.

En general, els nostres resultats validen l'eficàcia d'aquesta metodologia multifacètica per descobrir el paper dels gens candidats en la modulació de la presentació de l'esquizofrènia. En concret, hem identificat variants genètiques de *NRN1* associades a un risc més alt de patir trastorns de l'espectre esquizofrènic d'inici precoç, amb portadors que presenten una activitat alterada a l'escorça prefrontal dorsolateral durant una tasca de memòria de treball. També hem observat que les interaccions epistàtiques entre *NRN1*, *BDNF*-rs6265 i *CACNA1C*-rs1006737 modulen de manera significativa la gravetat clínica i les característiques neuroanatòmiques, on la interacció entre *NRN1* i *BDNF* a l'escorça orbitofrontal lateral esquerra media completament els efectes sobre la psicopatologia general. En mostres cerebrals post-mortem, els pacients amb esquizofrènia tractats amb clozapina van mostrar una menor expressió de *NRN1* i diferències de metilació a l'escorça prefrontal en comparació amb pacients no tractats i controls, amb aquestes diferències de metilació correlacionades amb l'expressió de *NRN1* i influïdes per variants genètiques específiques. Finalment, hem evidenciat la rellevància clínica dels nostres descobriments, demostrant que la teràpia de rehabilitació cognitiva s'associa amb canvis en la metilació perifèrica de *NRN1*, amb una major metilació diferenciant els pacients que responen dels que no responen en diversos dominis cognitius, un efecte també influït per la variabilitat genètica de *NRN1*.

En conjunt, els resultats presentats en aquesta tesi subratllen com la comprensió del paper de gens específics implicats en la plasticitat sinàptica pot no només aprofundir en el coneixement de l'etiologia de l'esquizofrènia, sinó també obrir vies per a la millora de l'atenció als pacients. Tot i les limitacions dels estudis centrats en gens candidats, derivades de la naturalesa poligènica del trastorn, aquests treballs aporten evidències sòlides i valuoses perspectives clíniques, establint les bases per a una investigació més àmplia d'aquests gens clau dins de xarxes moleculars complexes. Així, aquesta tesi representa una contribució significativa al procés col·lectiu de construcció de coneixement sobre l'etiologia de les psicosis, amb l'objectiu d'entendre com les variacions genètiques que afecten el neurodesenvolupament i la plasticitat sinàptica poden contribuir a les causes subjacents de l'esquizofrènia.

Paraules clau

Trastorns psicòtics, plasticitat sinàptica, genètica, metilació, *NRN1*

Table of contents

1.Introduction

1.1. The neurodevelopmental framework of schizophrenia

The human brain is an extraordinarily complex structure, containing approximately 10^{11} neurons spread across subcortical regions and the cortex. These neurons form grey matter with their cell bodies and white matter with their myelinated axons. Additionally, the brain is composed of around 10^{12} glial cells, which provide support and protection through astrocytes and microglia or insulate axons via oligodendrocytes. However, the true intricacy of the brain lies not merely in the number of its components but in the extensive network of neuronal connections, comprising an estimated 10^{15} synapses (Herculano-Houzel, 2009). This remarkable complexity requires over two decades to reach full maturity, shaped by a combination of genetic and environmental influences.

The neurodevelopmental processes coordinate how the brain acquires its intricate structure and interconnected networks (Silbereis et al., 2016) (Figure 1). The cortex grows rapidly from four postconceptional weeks to the third postnatal year, outpacing all other organ systems (Gogtay et al., 2004). It begins with neurulation, forming the neural tube, which segments into the forebrain, midbrain, and hindbrain (Donkelaar et al., 2023). In the dorsal telencephalon, neuroepithelial cells become radial glial cells (RGCs), initiating neurogenesis (Betizeau et al., 2013; Penisson et al., 2019). These cells produce neurons and intermediate progenitor cells in the subventricular zone, generating excitatory projection neurons that migrate and differentiate into the six-layered cortex through lamination (Hatanaka & Hirata, 2020; Mira & Morante, 2020). Meanwhile, cells from the caudal ganglionic eminences in the basal telencephalon become inhibitory interneurons, migrating tangentially to the cortex (Faux et al., 2012; Llorca & Marín, 2021; Zimmer-Bensch, 2018). As neurons migrate, they undergo neuritogenesis, extending dendrites and axons, and synaptogenesis, forming complex networks (Alfadil & Bradke, 2023; Lefebvre et al., 2015). Post-neurogenesis, RGCs give rise through gliogenesis to astrocytes and oligodendrocytes, while epithelial cells differentiate into microglia, all contributing to brain development (Molofsky et al., 2012; Tilborg et al., 2017). After this period, the rate of growth slows and processes such as synaptic maturation, dendritic and synaptic pruning and myelination predominate, mediated by the convergence of influences from intrinsic and extrinsic factors (Faust et al., 2021; Mordelt & de Witte, 2023; Seng et al., 2022; Spear, 2013; Tooley et al., 2021; Williamson & Lyons, 2018).

Notably, human neurodevelopment has unique features, as highlighted by comparative studies, which show that while ontogenetic patterns, neurogenic expression, cytoarchitecture, and cell-type composition are largely consistent across species, differences arise in the temporal and spatial gene expression trajectories of neurodevelopmental genes in humans (Zhu et al., 2018). During embryonic development, the human brain has a larger population of RGCs with increased proliferative capacity and prolonged neurogenesis, resulting in an overrepresentation of pyramidal cells in cortical layers II and III, which support human-specific cognitive and motor abilities (Dehay et al., 2015; Workman et al., 2013). Additionally, the human brain experiences

extended synaptogenesis, peaking from late prenatal stages to three years postnatally (Petanjek et al., 2011). Both processes contribute to human-specific cortical expansion. Following this period, synaptic refinement continues due to prolonged myelination and extensive adolescent synaptic pruning, both extending into the third decade, especially in associative regions of the frontal cortex (D. J. Miller et al., 2012). This is supported by imaging studies that have evidenced continued brain development through childhood, adolescence, and early adulthood, with differential trajectories related to grey and white matter. For example, while grey matter volume peaks in childhood and decreases through the second decade of life, white matter volume increases until mid-to-late adolescence before decelerating (Gogtay et al., 2004).

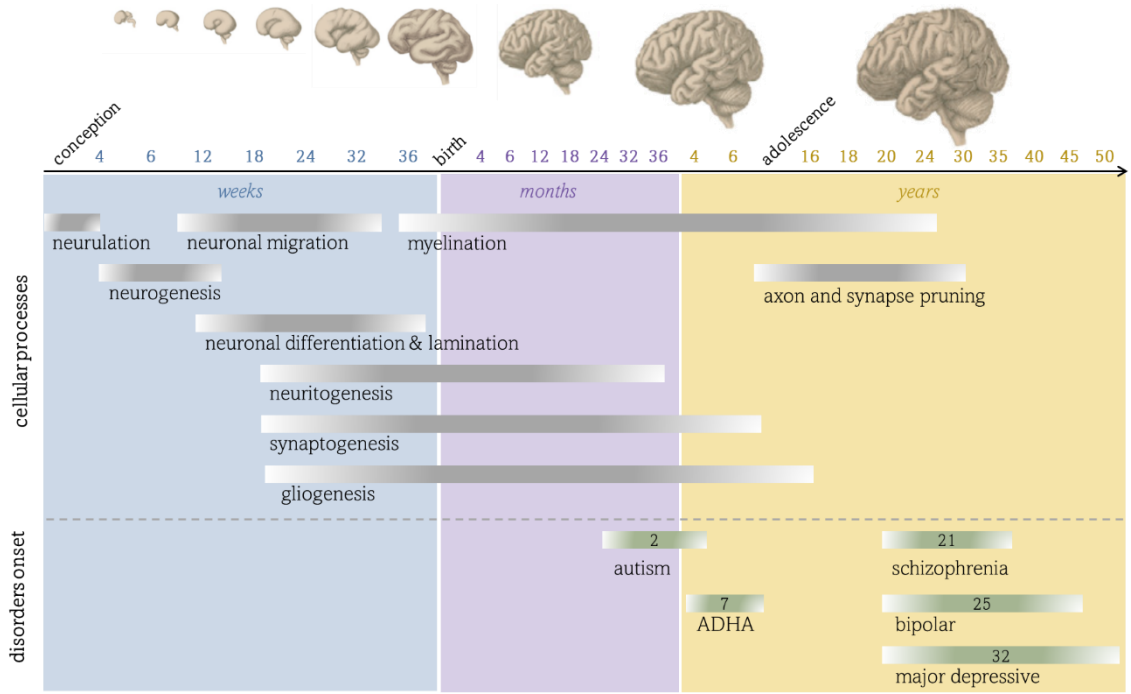


Figure 1. A representation of brain development and maturation in relation to the onset of schizophrenia. The figure illustrates, at the top, the structural changes occurring in the brain, while at the bottom, it displays key cellular processes and the average onset age for various mental disorders. These events are mapped to their estimated timing and sequence, spanning from conception through embryonic and fetal development (measured in weeks) to postnatal stages, including infancy (in months), childhood, adolescence, and adulthood (in years). Adapted figure (Silbereis et al., 2016).

These intricate molecular and cellular processes involved in human brain development are encoded within the complex genome (Zhou et al., 2024). Transcription serves as the initial step in translating genetic information into specific phenotypes, establishing distinct molecular and cellular characteristics. At this stage, the epigenome plays a crucial role in regulating spatiotemporal gene expression patterns (Bacon & Brinton, 2021; Lister et al., 2013; Murao et al., 2016; Sanosaka et al., 2009). Additionally, these epigenetic mechanisms can also be influenced by various external factors, serving

as a molecular bridge between environmental cues and gene expression (Perera et al., 2020). Remarkably, the great majority of protein-coding genes, along with an increasing number of non-coding genes, are active at some stage of brain formation (Kang et al., 2011). In terms of temporal expression, gene activity during prenatal and early postnatal periods accounts for nearly two-thirds of global expression variance, whereas changes in adulthood contribute only minimally (Kang et al., 2011). Regionally, co-regulated gene networks are expressed across brain areas during key developmental stages. These include genes involved in neuronal specification, which are highly active during embryonic and early fetal stages, and those related to synaptic function and ion channels, which peak in late fetal development and stabilize in early childhood (Kang et al., 2011). In the adult human brain, most genes in the brain are not limited to a single region but are instead active in multiple brain areas, with varying expression levels across those regions, suggesting that genes serve multiple functions, impacting different aspects of brain activity (Negi & Guda, 2017). However, transcriptomes show greater variability over time and across regions than across sexes, ethnicities, or individuals (Mazin et al., 2013).

The extended developmental window of the human brain enables extensive shaping by intrinsic and extrinsic factors, resulting in a structure and function that support the inherent variability in cognition, behavior, and personality traits observed in humans. However, this prolonged development also increases the brain's susceptibility to disruptions. The challenge of regulating diverse molecular and cellular processes over a long period and across various cell types and regions is evident in the distinct regional and temporal vulnerabilities of the brain to diseases, including schizophrenia. Thus, understanding this disorder requires a focused examination of the brain.

1.2. Diagnostic and clinical characteristics of schizophrenia

Two main diagnostic classifications

Although schizophrenia is recognized as a biological illness, no single brain characteristic, biomarker, or symptom definitively identifies it. The term *disorder* captures its complexity, as no specific cause has been established. Thus, the diagnosis still relies heavily on clinical evaluations, symptom assessment, and patient-reported experiences and history. However, with the intention of establishing a formal framework for identifying mental disorders, various guidelines have been developed, grounded in the conceptualization that psychopathology can be categorized into different disorders. This approach has led to the creation of diagnostic classification systems, such as the International Classification of Diseases (ICD) developed by the World Health Organization (WHO) and the Diagnostic and Statistical Manual (DSM) developed by the American Psychiatric Association (APA). These guidelines outline the specific clinical manifestations of each disorder, determined through expert consensus, providing a structured guide to diagnosis while reflecting evolving understandings of mental health.

In these nosologies, schizophrenia is currently diagnosed based on key symptoms, where the specific profile, severity, and duration are crucial not only for accurate diagnosis but also for understanding the individual's experience and tailoring the management and prognosis of the condition. In the current versions, DSM-5 and ICD-11, schizophrenia is diagnosed when at least two core symptoms are present. These include delusions, which are strongly held false beliefs that defy logical reasoning; hallucinations, where a person experiences sensory perceptions, such as hearing voices, that aren't present; and disorganized thinking (referred to as disorganized speech in DSM-5), which reflects chaotic or incoherent thought patterns that often result in fragmented or tangential communication. Additionally, both manuals also contemplate grossly disorganized or catatonic behavior, characterized by unpredictable actions, abnormal motor behavior, or immobility, and negative symptoms, such as diminished emotional expression, reduced motivation, and social withdrawal, which complicate daily functioning. Still, the two main diagnostic classification systems differ in symptom duration and focus on functionality. The DSM-5 requires six months of symptoms, with one month of active symptoms, and emphasizes functional impairment in areas like work, relationships, and self-care. In contrast, the ICD-11 requires only one month of symptoms, with less focus on functional impairment. Besides, while both systems have eliminated traditional subtypes, the DSM-5 emphasizes illness severity and progression with detailed specifiers, whereas the ICD-11 takes a more streamlined approach, focusing on the current symptom profile (Valle, 2020).

The heterogeneous course and phenotype

Schizophrenia usually emerges in late adolescence or early adulthood, peaking around age 20-21 (Solmi et al., 2022), though premorbid and prodromal signs often appear earlier, and presents a progression highly variable (**Figure 2**).

Premorbid developmental delays often appear much earlier, with neurological soft signs like motor skill delays (e.g., lifting the head, sitting, walking) and speech problems evident in the first year of life (Bachmann & Schröder, 2018; Petrescu et al., 2023). As individuals progress into childhood and adolescence, cognitive deficits and academic difficulties, including repeating grades, become more common (Fuller et al., 2002; Kendler et al., 2016; MacCabe et al., 2008; van Oel et al., 2002). Furthermore, a lower intelligence quotient by age 13 or a decline between ages 13 and 16 significantly increases the schizophrenia risk by 3.8% for each 1-point drop (Dickson et al., 2012; Khandaker et al., 2011; Woodberry et al., 2008). This is often accompanied by impairments in executive function, attention, memory, verbal episodic memory, and particularly processing speed, which plays a key role in mediating other cognitive abilities (Czepielewski et al., 2015; Meier et al., 2014; Reichenberg et al., 2010; Sheffield et al., 2018).

Besides, in the prelude to the disease, around 70% of individuals with schizophrenia go through a prodromal phase lasting one to five years, marked by a wide range of

symptoms, including attention difficulties, fatigue, anxiety, and anhedonia, along with more specific signs like perplexity, unusual beliefs, guardedness, and hearing vague or indistinct noises (Hafner et al., 2003; Marshall et al., 2014). Responses during this period vary significantly, with some individuals becoming erratic, while others withdraw (George et al., 2017). Additionally, many experience comorbidities such as depression, anxiety, and substance use, and about half notably struggle with suicidal ideation or attempts (Andriopoulos et al., 2011). As a result, the prodrome is recognized as a period of heightened vulnerability to psychosis. Although only one-third of those in the prodromal phase progress to full psychosis, the remainder either continue to experience attenuated symptoms with functional impairment or achieve remission (Wiltink et al., 2015). Importantly, around 80% of those who transition develop schizophrenia-spectrum disorders, while the rest are diagnosed with mood-related or atypical psychosis (Yung et al., 2005).

Evidence of developmental delays in both the premorbid and prodromal phases of schizophrenia suggests that genetic or environmental factors may disrupt essential neurodevelopmental processes, leading to altered brain development. Supporting this, research on ectodermal derivatives, such as facial features and skin, shows higher rates of minor physical anomalies and abnormal dermatoglyphic patterns in these individuals (Golembo-Smith et al., 2012; McGrath et al., 2002; Sut et al., 2024).

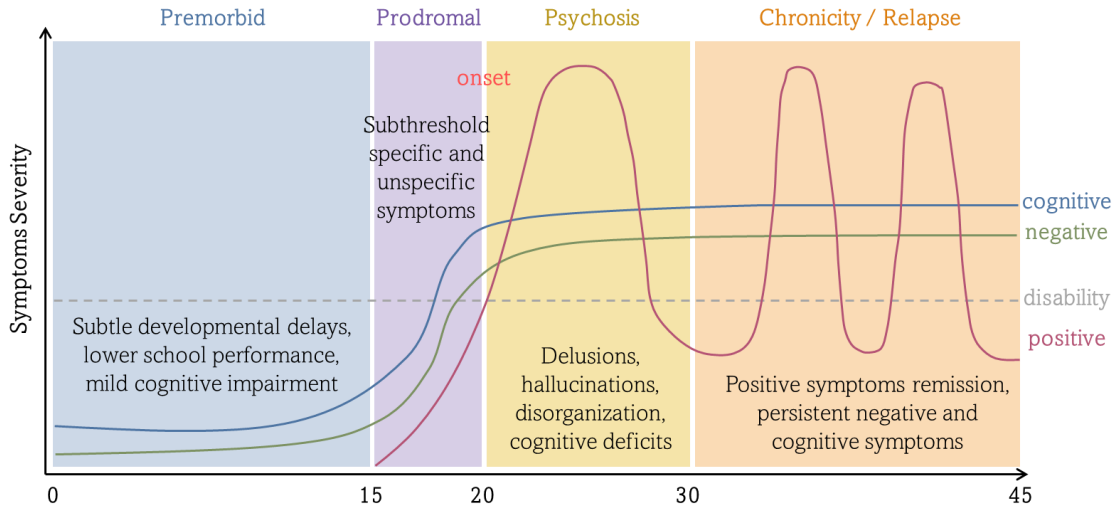


Figure 2. A schematic representation of the course of schizophrenia. The figure shows the premorbid and prodromal phases, with and the gradual worsening of cognitive (blue) and negative symptoms (green), followed by the onset characterized by an exacerbation of psychotic symptoms (pink). Adapted figure (McCutcheon et al., 2020).

After schizophrenia diagnosis, the condition presents in heterogeneous phenotype with diverse symptom patterns that vary significantly among individuals, as well as in different course trajectories with symptoms fluctuating in time both within the same person and across patients (Picardi et al., 2012).

Although the DSM-5 and ICD-11 describe schizophrenia through a range of main symptoms without formally dividing them into specific categories, these symptoms are often grouped into distinct types (**Figure 3**). These include, negative symptoms and disorganization, already mentioned and included per se in the manuals, plus positive symptoms, which encompass abnormal experiences such as delusions, hallucinations, suspiciousness, and grandiosity that distort a person's perception of reality (Andreasen et al., 1995). As a result of this heterogeneous presentation, to assess the symptom profile and severity in schizophrenia patients, healthcare professionals use clinical scales such as the Positive and Negative Syndrome Scale (PANSS), which helps monitor clinical status and track progression over time (Kay et al., 1987). In addition, although psychosis has traditionally been seen as the hallmark of schizophrenia, recent perspectives increasingly recognize cognitive impairment as a central feature of the disorder (Kahn & Keefe, 2013). Schizophrenia patients consistently exhibit poorer cognitive performance, yet it remains unclear whether these deficits worsen after the diagnosis. However, patients typically perform about 2 standard deviations below healthy controls, a significant drop compared to the 0.5 standard deviation seen before psychosis onset (Keefe et al., 2011). Additionally, most of the patients underperform relative to their mothers' educational levels (Jundong et al., 2011). Cognitive deficits affect multiple domains, with processing speed being particularly impacted and serving as a key predictor of overall cognitive function (Ojeda et al., 2012). Notably, these cognitive impairments are distinct from positive and negative symptoms and, while linked to disorganization, account for only a small portion of the overall variance (Moura et al., 2021). As these deficits are also presented in a highly heterogeneous way, to assess cognition clinicians and researchers use different scales, as for example the MATRICS Consensus Cognitive Battery (Kern et al., 2008; Nuechterlein et al., 2008).

One key aspect that influences the phenotypic manifestation and course of the disorder is the age at onset. Schizophrenia patients with early age at onset showed lower probability of symptomatic remission (Juola et al., 2013), more negative symptoms (Díaz-Caneja et al., 2015), and consequently more hospital admissions after over 10 years since onset (Rabinowitz et al., 2006).

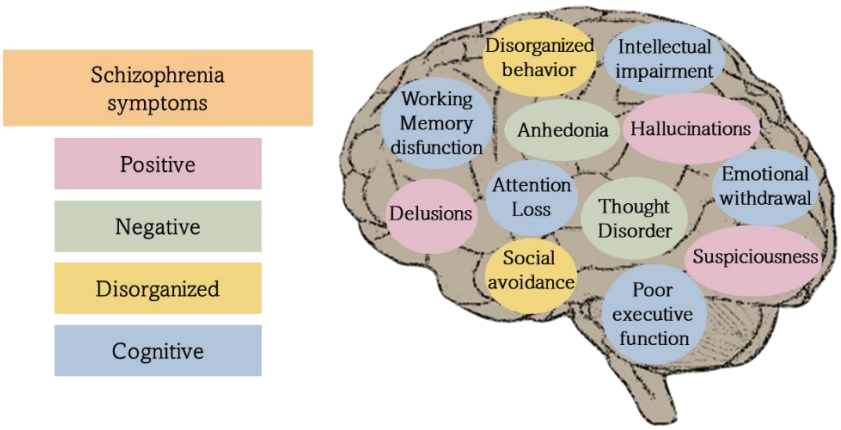


Figure 3. An illustration depicting the wide range of schizophrenia symptoms and its classification. The figure shows the classification of symptoms into four major dimensions: positive symptoms (pink), negative symptoms (green), disorganized behaviors (yellow) and cognitive impairments (blue).

The highly heterogeneous nature of schizophrenia, reflecting its complex etiology and the intricate nature of the human brain, has made it difficult to identify definitive biomarkers (Ahmed et al., 2018). Additionally, evidence shows that psychotic symptoms exist on a spectrum, ranging from subclinical to clinically significant, and are shared by related conditions such as schizophreniform disorder, schizoaffective disorder, and other unspecified psychotic disorders (Barr et al., 2022; Plana-Ripoll et al., 2019). This challenges the traditional view of mental disorders as distinct categories, instead suggesting they exist on a continuum (DeRosse & Karlsgodt, 2015; Owen, 2015). As a result, psychotic disorders are now seen as different manifestations of the same underlying processes, collectively referred to as schizophrenia-spectrum disorders (Liu et al., 2024; Romero et al., 2022).

To explore this perspective, the United States National Institute of Mental Health launched the Research Domain Criteria (RDoC) project (Kozak & Cuthbert, 2016), promoting research based on core functional domains rather than traditional diagnostic categories. Studies performed under the RDoC criteria examine behavior along a continuum from normal to pathological without rigid disorder boundaries, offering insights into the transition from typical to disordered behavior (Cuthbert & Morris, 2021). However, RDoC is a research tool, not a diagnostic system, intended to inform future classifications. Therefore, in the absence of a neurobiologically grounded classification system, the DSM-5 and ICD-11 remain essential, offering high utility by providing better diagnostic agreement and improving communication, including statistical reporting on psychiatric morbidity, services, treatments, and outcomes, which in turn has translated into valuable information about etiology (Clark et al., 2017; Kendell & Jablensky, 2003).

1.3. Epidemiology, social features and burden of schizophrenia

According to these diagnostic criteria and data from the 2019 Global Burden of Disease (GBD) study, the global prevalence of schizophrenia, defined as the total number of cases within a population at a specific time, is estimated at 23.6 million (95% UI: 20.20–27.10). Meanwhile, the incidence, which represents the number of new cases occurring within a given period, is estimated at 1.29 million worldwide (95% UI: 1.09–1.53). These numbers reflect increases of 65.87% and 37.11% in prevalence and incidence, respectively, since 1990. While these increases in absolute numbers may appear striking, when adjusted for population age structure these estimates have remained relatively stable, with global age-standardized prevalence at 287.41 per 100,000 (95% UI: 246.16–330.88) and global age-standardized incidence at 16.31 per 100,000 (95% UI: 13.80–19.42), reflecting slight decreases of 0.87% and 3.30%, respectively, over the same period (GBD 2019 Mental Disorders Collaborators, 2022; Solmi et al., 2023). Although the diagnostic criteria shifted from DSM-4 to DSM-5 during this period, this change appears to have had minimal impact on overall prevalence (Tandon et al., 2013).

These estimates show regional differences, which can be explained by two main factors. First, high-income countries have well-developed health systems that routinely collect and store mental health data, providing a clearer picture of prevalence (Saraceno et al., 2007). Second, variations arise from specific social determinants in each region, creating constellations of environmental risk factors (Lund et al., 2018).

The question of whether schizophrenia is more common in men or women remains debated, with some studies indicating no major sex differences in prevalence, while others report a slightly higher incidence among men (Charlson et al., 2018; Jongsma et al., 2019). However, distinct patterns in onset are evident, as men generally experience a peak in their early twenties followed by a decline, whereas women show a less pronounced peak with a gradual decrease and see new cases surpassing those in men after age 45 (Selvendra et al., 2022). Additionally, research indicates that symptom profiles may vary by sex, with men tending to experience more prominent negative symptoms and women more likely to display affective symptoms, including depression, impulsivity, and emotional instability (Leger & Neill, 2016).

People with schizophrenia have, on average, a shorter lifespan, with a life expectancy gap of 15–20 years, and a higher mortality rate than the general population, experiencing 3 times higher all-cause mortality (Correll et al., 2022). Moreover, schizophrenia patients face the greatest mortality risk among all psychiatric disorders (Correll et al., 2022). Many factors contribute to sustaining this higher rate of mortality. First, the lifetime prevalence of suicide attempts and suicide death are 27% and 5% respectively, which represents more than 10 times higher suicide mortality rates than the general population (Hor & Taylor, 2010; Lu et al., 2019). Second, unhealthy lifestyle habits like smoking, alcohol consumption, poor diet, and lack of physical activity are more common among individuals with schizophrenia compared to the general population. These behaviors also

contribute to a higher incidence of cardiovascular disease and diabetes mellitus (Heald et al., 2017; Kalinowska et al., 2021; Ratliff et al., 2012). Moreover, antipsychotic medications are linked to an increased risk of diabetes, hypertension, high cholesterol, metabolic syndrome, and liver damage (Pillinger et al., 2020). Interestingly, despite these side effects, antipsychotic use has been associated with reduced mortality, indicating that their benefits may outweigh the risks (Jia et al., 2022).

Moreover, schizophrenia is notable among mental health disorders for its tendency to evoke predominantly negative attitudes from the general population, contributing to a public stigma rooted in stereotypes and prejudices that lead to the perception that individuals affected by the disorder lack the capacity for social interaction. Extensively studied aspects of this stigma include perceptions regarding danger, inclination towards social distance, and avoidance tendencies (Fond et al., 2023; Mannarini et al., 2022; Zamorano et al., 2023). These societal beliefs can create barriers for individuals with schizophrenia, impeding their access to suitable housing and opportunities for employment and education, ultimately resulting in social exclusion and high suicidality among these individuals (Hipes et al., 2016; Lincoln et al., 2021; Sharaf et al., 2012).

The impact of these clinical and social factors on the population is measured using Disability Adjusted Life Years (DALYs), which account for both Years Lived with Disability (YLDs) and Years of Life Lost (YLLs) due to premature death (**Figure 4**). In 2019, mental disorders were the seventh leading cause of DALYs globally, contributing 125.3 million (95% UI: 93.0–163.2), or 4.9% (3.9–6.1) of the global burden, with an age-standardized rate of 1,566.2 per 100,000 people (95% UI: 1,160.1–2,042.8). Schizophrenia alone accounted for 12.2% of these DALYs, ranking third among mental disorders. The raw DALY estimate for schizophrenia was 15.1 million (95% UI: 11.00–19.21), reflecting a 65.44% increase since 1990. However, age-standardized DALYs have remained relatively stable at 184.15 per 100,000 people (95% UI: 134.32–234.54). It's noteworthy that DALYs for mental disorders are primarily driven by non-fatal burden, as they are mostly composed of YLDs. From 1990 to 2019, mental disorders were the second leading cause of YLDs globally, contributing 125.3 million (95% UI: 93.0–163.2), or 14.6% (12.2–16.8) of global YLDs in 2019. Although schizophrenia ranks twentieth globally for YLDs, its impact varies with age, being the ninth leading cause among those aged 25 to 49, coinciding with its typical onset in early adulthood (GBD 2019 Mental Disorders Collaborators, 2022).

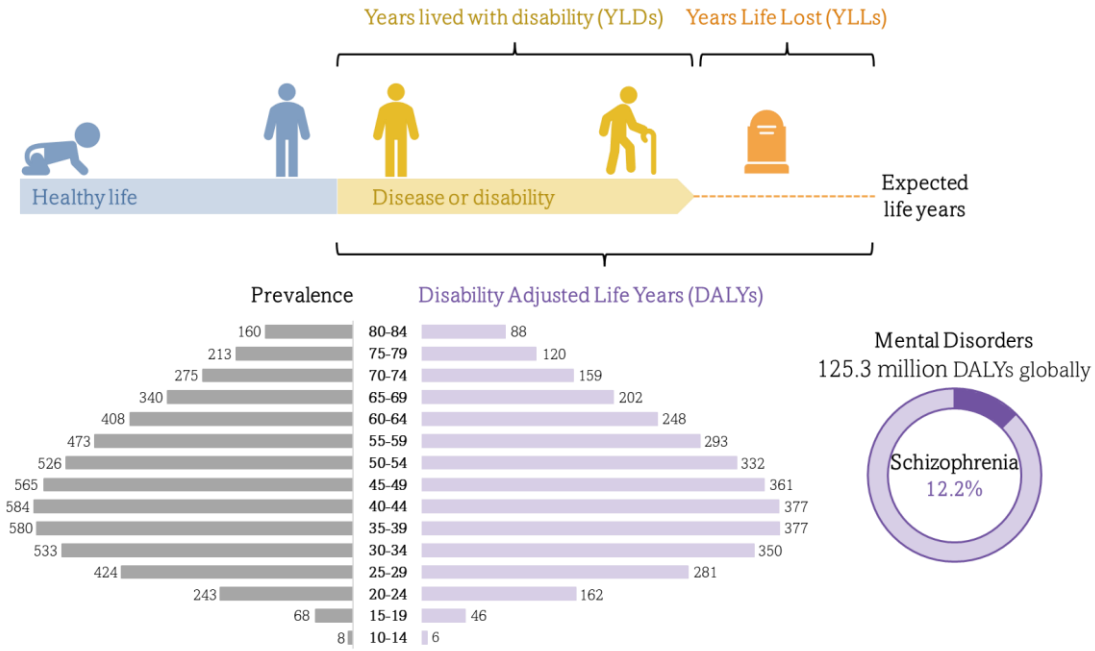


Figure 4. A schematic representation of burden metrics and their relation to schizophrenia. The figure illustrates, at the top, the method used to calculate burden metrics, such as Disability Adjusted Life Years (DALYs), a measure that combines both years lived with disability (YLDs) and years of life lost due to premature death (YLLs). At the bottom, it shows the prevalence of schizophrenia and the distribution of DALYs across different age groups. Additionally, it presents the total DALYs estimated for all mental disorders and highlights the percentage attributable to schizophrenia.

1.4. Pathophysiology of schizophrenia: focus on synaptic plasticity

Schizophrenia symptoms can be understood as the difficulty of the brain in regulating goal-directed behavior and integrating complex sensory information. These diverse symptoms are likely connected to the processes that shape brain structure and function.

In the human brain, neurons interact through synapses, which are composed of a presynaptic terminal, a postsynaptic structure, and the synaptic cleft between them (Holler et al., 2021). Throughout life, from birth to adulthood, brain development depends on synaptic plasticity, the process by which neurons modify their connections in response to external signals, refining synapses and creating neural circuits (Marzola et al., 2023). Plasticity depends on neuronal excitability, with neurons generating action potentials in response to stimuli, causing temporary changes in the electrical charge of the neuronal membrane via voltage-gated ion channels (Ma et al., 2023). The brain relies on two types of synaptic plasticity to support memory and behavior: short-term and long-term plasticity. Short-term plasticity, lasting milliseconds to minutes, plays a crucial role in short-term memory and immediate behavioral responses. It is driven by an increase in presynaptic calcium levels, which enhances neurotransmitter release. Long-term plasticity, which involves persistent changes lasting hours to days, includes long-term potentiation (LTP) and long-term depression (LTD), both mediated by activity-dependent

signaling pathways. On the one hand, LTP, a strengthening of synapses, is essential for long-term memory storage. It is initiated by calcium influx through NMDA (N-methyl-D-aspartate) receptors, which increases calcium levels in dendritic spines. This activates signaling pathways that insert AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors into the postsynaptic membrane, enhancing synaptic efficacy and strengthening the connection (Magee & Grienberger, 2020). In contrast, LTD, a weakening of synapses, is critical for synaptic pruning, learning, and memory flexibility. It is triggered by prolonged low-frequency stimulation, which activates calcium signaling pathways that lead to the removal of AMPA receptors from the postsynaptic membrane, reducing synaptic efficacy (Cummings et al., 1996). Then, the spatiotemporal dynamics of the calcium signal, along with its overall amplitude determine the direction of the synaptic change, whether towards LTP or LTD. This ability of the brain to reorganize both structure and function allows neural networks to develop new skills while maintaining stability (Bassi et al., 2019). Synaptic plasticity is therefore essential for vital cognitive functions such as thinking, perception, learning, and memory, as it plays a key role in encoding and storing information. Moreover, it helps the brain adapt to challenges, highlighting its importance in maintaining cognitive health and resilience (Stuchlik, 2014).

Consequently, among the various theories that seek to explain the core pathophysiological changes in schizophrenia, one suggests that the disorder stems from impaired regulation of synaptic plasticity (Howes & Onwordi, 2023). This hypothesis posits that a defect in the prefrontal cortex emerges during early development, potentially due to inadequate synaptogenesis, and worsens during adolescence when excessive pruning of excitatory synapses reduces synaptic density. Feinberg first proposed this idea in 1982, noting that in healthy brains EEG wave amplitudes, neuronal metabolism, and neuroanatomical plasticity mirror synaptic density trajectories, which peak in childhood, decline during adolescence, and stabilize in adulthood. He observed that schizophrenia often begins during adolescence, a period of intense synaptic pruning that aligns with the peak of cognitive performance (Feinberg, 1982).

Since its early postulation, this theory has been supported by diverse evidence. On the one hand, histological studies have revealed significant synaptic abnormalities in schizophrenia patients compared to controls. For instances, pre-synaptic synaptophysin levels are lower in key brain regions, while post-synaptic elements, such as dendritic spine density and protein expression, are notably reduced, particularly in cortical areas (Berdenis van Berlekom et al., 2020; Osimo et al., 2019). Additionally, electron microscopy confirms fewer synapses, including axon-spinous and axon-dendritic synapses in the anterior cingulate cortex, reduced glutamatergic synapses in the striatum, and fewer inhibitory synapses in the substantia nigra (Aganova & Uranova, 1992; Kung et al., 1998; Mabry et al., 2020; McCullumsmith et al., 2014; Roberts et al., 2015). On the other hand, while the exact mechanism behind excessive pruning is still under investigation, animal models suggest that complement proteins like C3 and C4 tag redundant synapses for removal by microglia (Faust et al., 2021). Notably, human iPSC

(induced pluripotent stem cells) models also show increased complement-dependent synaptic elimination, indicating glial involvement (Sellgren et al., 2019). Besides, environmental factors such as maternal infections, obstetric complications, immune activation, trauma, and stress, all known schizophrenia risk factors, also activate microglia (Hanamsagar & Bilbo, 2017).

Beyond histological and in vitro models examining synaptic density and pruning mechanisms, early neurochemical studies indicate that synaptic plasticity alterations are also reflected in neurotransmitter system dysfunctions. On the one side, multiple lines of evidence link psychotic symptoms to dopaminergic neurons in the mesolimbic pathway, which under normal conditions signal the relevance of stimuli (Kesby et al., 2018; Luo & Huang, 2016). Dopamine, a neurotransmitter with both inhibitory and excitatory roles, is released into the synaptic cleft and binds to post-synaptic receptors (D1, D2, D3, D4, and D5) (Martel & Gatti McArthur, 2020). Pharmacological studies have shown that dopamine-releasing drugs like amphetamines can induce psychosis in healthy individuals and worsen symptoms in those with schizophrenia (McKetin et al., 2019; Rognli & Bramness, 2015). Similarly, molecular imaging reveals increased striatal dopamine synthesis and release in patients, even during the prodromal phase, with activity intensifying as the disorder progresses (Egerton et al., 2013; van Hooijdonk et al., 2023; Watanabe et al., 2014). Post-mortem studies also report higher expression of dopaminergic receptors (Reynolds, 2022). This overactivation of dopaminergic neurons has been proposed to explain positive symptoms of schizophrenia, though it does not fully account for negative or cognitive symptoms (Laruelle & Abi-Dargham, 1999). On the other side, dysfunctions in the glutamatergic neurotransmitter system and its receptor, the NMDA, the most abundant excitatory neurotransmitter in the brain, have also been observed (Zhou & Danbolt, 2014). This theory arose from observations that NMDA receptor antagonists, like phencyclidine and ketamine, can induce positive symptoms in healthy individuals, alongside findings of antipsychotic neuroprotection against NMDA-hypofunction-induced neurodegeneration in animal models (Beck et al., 2020; Villéga et al., 2024; Zorumski et al., 2016). Additionally, many other pieces of evidence from clinical, neuroimaging and post-mortem studies have provided evidence on NMDA hypofunction and glutamatergic neurotransmission abnormalities in the pathophysiology of schizophrenia (Kruse & Bustillo, 2022; Uno & Coyle, 2019). Even though these are the neurotransmitter systems with the strongest evidence in schizophrenia, other neurotransmitter types, including endocannabinoid and serotonin systems, have been associated with the disorder (Stahl 2018; Jauhar et al. 2022; Durieux et al. 2022).

In addition, synaptic plasticity dysfunction in schizophrenia can be interpreted through the lens of neuroanatomical and neurofunctional changes, which are widely documented in the disorder. Structural Magnetic Resonance Imaging (MRI) techniques (**Box 1**) have provided valuable insights into grey and white matter abnormalities in schizophrenia, with meta-analyses and large consortium studies clarifying global and region-specific brain alterations in schizophrenia. Common structural findings include ventricular

enlargement, increased cerebrospinal fluid volume, and reduced total brain and intracranial volumes, indicating a generalized loss of brain tissue. This is primarily driven by significant decreases in total grey matter volume, particularly in cortical regions, with smaller but notable reductions in white matter volume (Haijma et al., 2013; Kuo & Pogue-Geile, 2019). Subcortical changes include increased volumes in the globus pallidus and decreased volumes in the nucleus accumbens, hippocampus, amygdala, and thalamus (Haijma et al., 2013; Kuo & Pogue-Geile, 2019; van Erp et al., 2016). Cortical changes include significant grey matter volume reductions in the frontal, temporal, parietal, and occipital lobes, as well as in the cingulate gyrus and insula. The most pronounced volume reductions are seen in the frontal lobe, affecting prefrontal regions and specific areas such as the inferior, middle, and superior frontal gyri, and in the temporal lobe, including the superior temporal gyrus, fusiform gyrus, and planum temporal (Haijma et al., 2013; Kuo & Pogue-Geile, 2019). These structural alterations have been also described in drug-free and first-episode patients (Brugger & Howes, 2017; Glahn et al., 2008). Additionally, widespread cortical thinning and surface area reductions have been reported in patients, with the most significant effect sizes in frontal and temporal regions (Erp et al., 2018).

Alterations in grey and white matter contribute to dysfunctions in neural communication and overall brain function. In line with this, disruptions in synaptic plasticity are supported by functional MRI findings that have highlighted the impact of abnormal neuronal excitability. Meta-analysis of functional MRI studies using resting-state paradigms reported hypo-connectivity within the default-mode, affective, ventral attentional, thalamus and somatosensory networks (Dong et al., 2018). Additionally, functional brain abnormalities in schizophrenia have been identified across several cognitive domains, with a strong emphasis on working memory (Box 2). Meta-analyses of functional MRI studies investigating these tasks consistently reveal dysfunction in critical areas of the central executive and salience networks, such as the bilateral dorsolateral prefrontal cortex, posterior parietal cortex, anterior insula, anterior cingulate cortex, and supplementary motor area. Moreover, schizophrenia patients show deactivation abnormalities in core regions of the default mode network, including the ventromedial prefrontal cortex and posterior cingulate cortex (Wu & Jiang, 2020).

Although structural and functional MRI evidence does not represent a direct measure of synaptic plasticity alterations, some remarkable studies have suggested that changes in grey matter volume and cortical thickness reflect synaptic loss in schizophrenia (Keifer Jr et al., 2015; Nagasaka et al., 2021).

Box 1. Structural Neuroimaging

Principle

In an MRI scanner, a strong magnetic field aligns hydrogen protons in the body, and after a radiofrequency pulse disrupts this alignment, the protons emit energy as they return to their original positions, which is captured as MR signals to generate images of brain activity. Two structural images are generated, T1 (longitudinal relaxation time), that measures how quickly the protons realign with the magnetic field, and T2 (transverse relaxation time), that tracks how quickly they lose phase coherence (Grover et al., 2015).

Analysis

To analyze cortical morphology using T1 weighted MRI, two key methods are voxel-based morphometry (VBM) and surface-based morphometry (SBM). Both techniques segment grey and white matter into anatomical regions, but VBM focuses on estimating grey matter volume, while SBM offers detailed measurements of features like cortical thickness, surface area, and gyrification (Goto et al., 2022).

Software

FreeSurfer is a widely used tool for SBM, automatizating the segmentation of brain tissues, including grey matter, white matter, and subcortical structures, for volumetric analysis. It also reconstructs detailed cortical surface models for precise measurement of cortical features. Furthermore, FreeSurfer performs cortical parcellation using anatomical atlases, like the Desikan-Killiany, enabling standardized analysis of cortical regions across studies (Fischl, 2012).

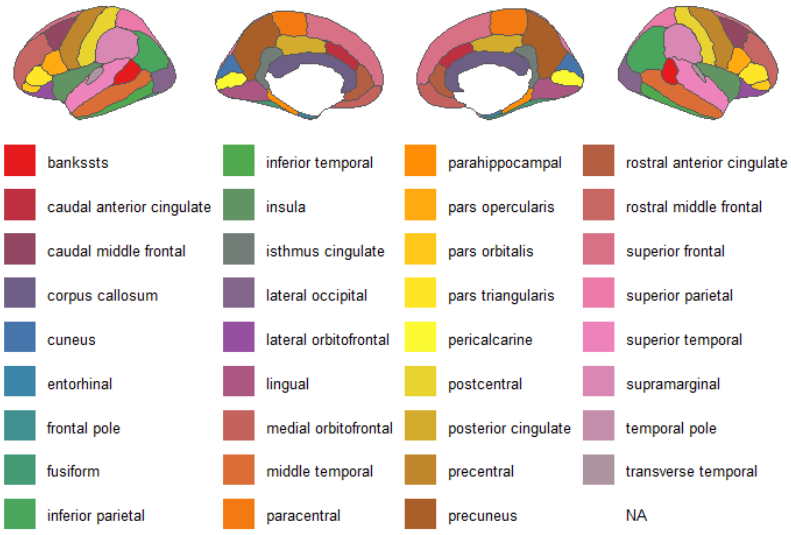


Figure B1. Representation of the different regions of interest (ROI) parcellated by the FreeSurfer software according to the Desikan-Killiany atlas. The figure displays, at the top section, left and right lateral and medial views of the brain highlighting regions of interest in color, and at the bottom section, it includes a legend with each region of interest labeled in its corresponding color. Image generated using the R packages ggplot and ggseg.

Box 2. Functional Neuroimaging

Principle

Like structural MRI, functional MRI (fMRI) uses a strong magnetic field and radio-frequency pulse to align hydrogen protons, capturing the energy they emit as MR signals when they realign. In this case, the capture is based on the Blood Oxygen Level-Dependent (BOLD) signal that is an indirect measure of brain activity. When neurons become active, local blood flow increases to supply more oxygen, which reduces the amount of deoxyhemoglobin compared to oxyhemoglobin. Deoxyhemoglobin, being paramagnetic, alters the magnetic field and affects the precession speeds of nearby protons, unlike oxyhemoglobin, which is diamagnetic. This alteration in precession speeds is detected by the scanner as a weaker T2 signal, which measures how quickly the protons lose phase coherence, compared to when the blood is oxygenated (Hillman, 2014).

Analysis

Notably, fMRI is commonly used to examine brain regions activated during different tasks using software like FSL. The BOLD signal at each voxel is modeled based on the task the subject is performing, with brain activity compared across different conditions (Smith et al., 2004).

N-back paradigm

A popular example is the N-back task, a working memory fMRI paradigm that varies in difficulty to increase memory load and engage attention and working memory processes. At lower difficulty levels, frontally centered networks are crucial for attention-based performance, while higher memory loads activate both frontal and parietal networks involved in working memory (Kane et al., 2007). This task is particularly valuable as it specifically assesses working memory, the capacity to retain and manipulate information for goal-directed behavior, which is strongly influenced by genetic factors, with heritability estimates between 43% and 49% (Blokland et al., 2011). Both patients with schizophrenia and their affected siblings exhibit deficits in this area compared to healthy individuals, resulting in significant impairments in daily functioning (Park et al., 1995; Wu & Jiang, 2020).

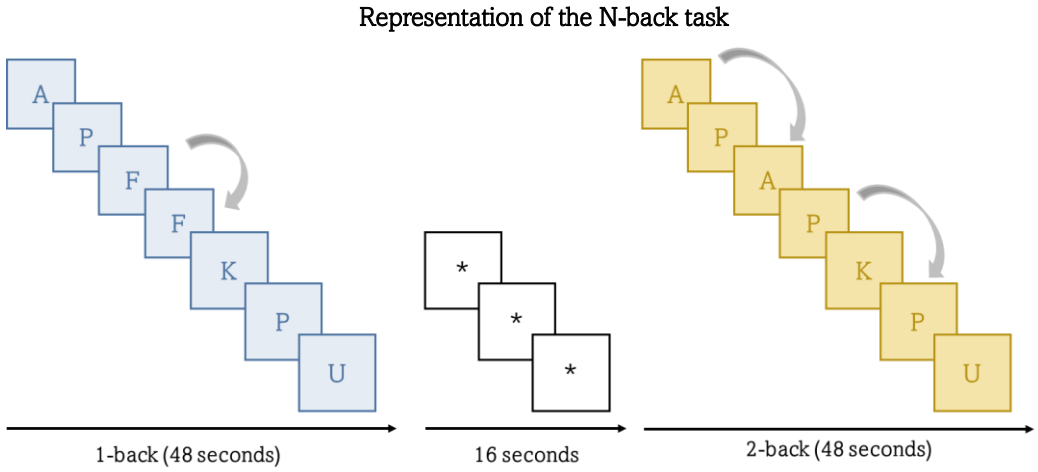


Figure B2. Representation of the sequential-letter version of the N-back task used in the fMRI paradigm of this thesis. The figure represents the objective of the task, which is to indicate letter repetitions. There are two memory load levels: the 1-back (most accessible level, in blue) and the 2-back (most challenging level, in yellow), presented in a blocked design manner. The whole task consists of four blocks for each level presented interleaved and separated by a baseline stimulus (asterisk), each containing five letter repetitions randomly located.

1.5. Treatment strategies for schizophrenia

Once diagnosed with schizophrenia, individuals may be treated with a combination of medication, psychological intervention, and community-based assistance.

Pharmacological interventions for schizophrenia are largely informed by the pathophysiological hypotheses of the disorder, particularly the role of dopamine dysregulation. Most antipsychotic drugs developed to date act as dopamine D2 receptor antagonists (Ginovart & Kapur, 2012). These medications are categorized into first-generation antipsychotics (FGAs), such as haloperidol and chlorpromazine, and second-generation antipsychotics (SGAs), including clozapine, risperidone, olanzapine, and quetiapine. While both FGAs and SGAs block dopamine D2 receptors, they differ in their binding affinities and receptor profiles (Siafis et al., 2021). Additionally, some SGAs also bind to receptors in the adrenergic, cholinergic, and histaminic systems, which contributes to their lower risk of extrapyramidal side effects (de Greef et al., 2011). Depending on the specific receptor profile, these medications can lead to side effects such as sedation (from histamine blockade), orthostatic hypotension (from adrenergic blockade), and symptoms like dry mouth, constipation, hyperthermia, and cognitive impairment (from cholinergic blockade) (Kapur & Remington, 2001).

Comparisons between SGAs and FGAs, as well as within each category, reveal no consistent differences in efficacy, except for clozapine, which has shown superiority in treatment-refractory schizophrenia, which affects about one-third of cases and significantly lowers their quality of life (Leucht et al., 2013). This drug acts as a dopamine receptor antagonist, showing low occupancy at D2 receptors and high affinity for D4, while also binding strongly to serotonin, adrenergic, histamine, and muscarinic receptors. Its rapid dissociation from D2 and antagonism of serotonin 5-HT2A (5-hydroxytryptamine) receptors likely contribute to its effectiveness, while its broad receptor interactions account for many of its side effects (Kapur & Seeman, 2001). Although the exact molecular pathways critical to its effectiveness are unclear, animal studies suggest that clozapine improves behavioral outcomes by modulating genes related to cholesterol metabolism, GABAergic function, cell cycle control, neurotrophins, and synaptic plasticity through reducing methylation or producing histone modifications (Guidotti et al., 2017). Meanwhile, human studies are constrained by limited access to post-mortem brain samples and the infrequent use of this treatment among patients. One study using publicly available schizophrenia transcriptomic data found that three genes (*GCLM*, *ZNF652*, *GYPC*) and four pathways involved in protein trafficking, neuronal migration, brain development, and synaptic function were consistently differentially expressed in clozapine-treated patients compared to those on other medications (Lee et al., 2017).

Although various factors influence the course and prognosis of the disorder, evidence highlights that adherence to pharmacological treatment is crucial during this period (Verdoux et al., 2000). The longer psychotic symptoms go untreated, the higher the risk

of relapses and significant clinical deterioration (Penttilä et al., 2014). Although the maximum effect, however, may not be achieved for several months, response over the first 2–4 weeks of antipsychotic therapy is highly predictive of long-term response (Kinon et al., 2010). Response to antipsychotics also depends on the illness stage, with first-episode patients typically showing quicker and higher response rates than those in later stages (Emsley et al., 2006).

Other non-pharmacological treatments have also shown promising results in enhancing symptoms and quality of life for patients. For instance, assertive community treatment, for example, have been linked to improvement of independent living and treatment retention, though it has been proved to have limited impact on core symptoms (Coldwell & Bender, 2007; Frederick & VanderWeele, 2019). Also, family interventions have been associated with reduce relapse rates, though they have been shown to have minimal effect on other outcomes (Kim & Park, 2023). Besides, illness self-management, psychoeducation, and social skills training have been related to decrease symptom severity and relapse rates, improving social and global function, each adding valuable support to patient care (Lean et al., 2019).

Among behavioral interventions, cognitive therapies, including cognitive behavioral therapy and cognitive remediation, are the most recommended evidence-based approaches for schizophrenia, offering benefits in enhancing global function, quality of life, and core symptoms, through their long-term effects continue to be an area of active research (Berendsen et al., 2024). Specifically, cognitive remediation, which can be delivered via a therapist-supported web-based program like CIRCuiTS (Computerized Interactive Remediation of Cognition and Thinking Skills; Reeder & Wykes, 2010), has shown particular benefits for individuals with more severe clinical impairment (Wykes et al., 2002). Additionally, its efficacy has been supported by its capacity to modulate brain function and structure, neuroimaging studies consistently reveal increased bilateral activation in prefrontal and parietal areas as the most common finding associated with CRT (Mothersill & Donohoe, 2019; Penadés et al., 2017). Furthermore, changes in neuronal-connectivity (Penadés et al., 2020; Ramsay et al., 2017), along with structural modifications in white matter (Matsuoka et al., 2019; Penadés et al., 2013) and grey matter (Eack et al., 2010; Morimoto et al., 2018), have been observed. Interestingly, these functional and structural changes correlated with behavioral improvements in most of the mentioned studies.

It is crucial to acknowledge that current treatments rarely result in a complete cure. As mentioned, a substantial proportion of patients may show limited responsiveness, with cycles of relapse and remission, and some may experience a progressive worsening of symptoms. While positive symptoms tend to receive considerable focus due to their visibility and often show improvement with antipsychotic medications and psychological interventions, it is the negative and cognitive symptoms that most substantially contribute to the overall morbidity and functional impairment associated with schizophrenia

(Carbon & Correll, 2014). Therefore, it has been suggested that the most effective treatment combines pharmacological approaches, such as antipsychotics, with cognitive or psychological interventions.

1.6. Etiological factors of schizophrenia

Although the underlying causes and mechanisms of schizophrenia remain largely unknown, substantial evidence suggests that it is a complex and multifactorial origin resulting from both genetic and environmental factors as well as their interaction through epigenetic mechanisms that, in a coordinated manner, orchestrate brain development and maturation (**Figure 5**).

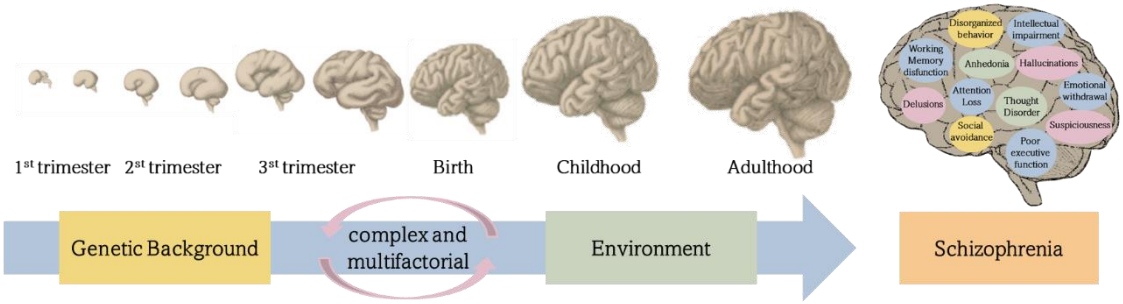


Figure 5. An illustration depicting the complex and multifactorial etiology of schizophrenia. The top section of the figure highlights structural changes in the brain, while the bottom features an arrow representing the timeline of brain development, shaped by the interplay between genetic factors and environmental influences. Adapted figure (Silbereis et al., 2016)

Environmental factors

Epidemiological studies have identified many environmental factors associated with the increased risk of schizophrenia, suggesting a complex picture with a multitude of social, physical and chemical exposures occurring at different stages of life (Stilo & Murray, 2019).

A controversial factor in schizophrenia risk is parental age. Increased paternal age, starting from over 34, has been linked to the disorder, possibly due to age-related mutations in male germ cells, although personality traits leading to delayed marriage and reproduction may also play a role (Janecka et al., 2017; Khachadourian et al., 2021). In contrast, maternal age, particularly younger than 19 or older than 40, has similarly been associated with an elevated risk of schizophrenia in offspring (Byrne et al., 2003; Ni et al., 2018).

One vulnerability window for schizophrenia occurs during the prenatal and perinatal periods (Schmitt et al., 2023). Common obstetric complications associated with an

increased risk of schizophrenia include bleeding, diabetes, rhesus incompatibility, pre-eclampsia, and uterine atony. Additionally, emergency cesarean sections, placental abruption, and perinatal hypoxia further elevate the risk (Radua et al., 2018). All these events do not have a dismissible risk, as the pooled odds ratio is around 2 (Belbasis et al., 2018). Similarly, maternal stress and infections have also been linked to higher risk, such as people born in winter that are more exposed to maternal respiratory infections or to maternal malnutrition, including folic acid or vitamin D deficiency, occurring during these months (Pugliese et al., 2019). These complications can disrupt normal fetal growth and development, increasing the risk of schizophrenia and leading to outcomes such as prematurity, low birth weight (odds ratio ~3.2), small head circumference (odds ratio ~1.6), and congenital malformations (odds ratio ~2–2.5) (Harper et al., 2015; Rubio-Abadal et al., 2015). However, long-term consequences of prenatal and perinatal hazards include not only association with psychosis but also with a range of cognitive deficits and neurological abnormalities as well as other psychiatric and neurodevelopmental disorders (Amoretti et al., 2022).

Childhood is another susceptibility period for schizophrenia, as adversities like sexual, physical, emotional, or psychological abuse, neglect, parental death, or bullying are linked to a significantly higher risk of developing psychosis in adulthood (odds ratio ~ 2.8) (Varese et al., 2012). These childhood adversities are especially associated with more severe positive symptoms in adulthood, such as hallucinations and affective symptoms (Trotta et al., 2015).

Other factors are also associated with an increased risk for schizophrenia. Demographic factors, like migration status, are associated with an increased risk of psychosis, though this risk varies significantly depending on factors such as country of origin and destination, reasons for migration, and age at migration (Bourque et al., 2011; Leaune et al., 2019). Economic factors also play a role, with countries experiencing greater economic disparity showing higher incidence rates of schizophrenia (Burns et al., 2014). Conversely, employment serves as a protective factor, linked to better social functioning, less severe symptoms, improved quality of life, and higher self-esteem for those living with schizophrenia (Luciano et al., 2014). Moreover, neighborhood environments, such as low socioeconomic status, community instability, and urban living, are associated with a higher prevalence of psychotic disorders and reduced cognitive function in older adults (Fett et al., 2019; O'Donoghue et al., 2016; Wu et al., 2015). Meanwhile, racial segregation has mixed effects, as it can exacerbate social disparities but, at the same time, high-density ethnic communities may provide increased social support, offering protective benefits (Bosqui et al., 2014). Finally, personal environments, including social capital and networks, are often diminished in individuals with psychotic disorders, not only because of social exclusion due to their condition but also as a contributing factor (Silva et al., 2005).

Genetic factors

To investigate the influence of genetic and environmental factors in the development of schizophrenia, researchers have employed family, adoption, and twin studies, which have provided valuable insights into the relative contributions of each factor to the disorder.

Family studies, which aim to investigate whether a condition clusters among family members across generations, have shown that schizophrenia is more prevalent among the relatives of diagnosed patients compared to the general population (Henriksen et al., 2017) (**Figure 6**). More specifically, they have indicated that the risk of developing the disorder increases with the degree of familial relatedness to a proband, meaning that a higher degree of shared genetics corresponds to an increased likelihood of the condition (Gottesman, 1991). For instance, when the affected family member is a first-degree relative, such as a parent or sibling, the relative risk is between 9.0 and 9.9. In contrast, for second-degree relatives, such as half-siblings, the relative risk is between 2.7 and 3.6 (Lichtenstein et al., 2009). A recent meta-analysis further supports these findings, estimating that the risk of developing schizophrenia increases eightfold for first-degree relatives compared to the general population (Le et al., 2020). Interestingly, this risk is not limited to schizophrenia, as having a psychotic disorder increases the likelihood that relatives will develop the same or another psychotic condition, thus supporting the idea of the psychotic continuum (Sandstrom et al., 2019).

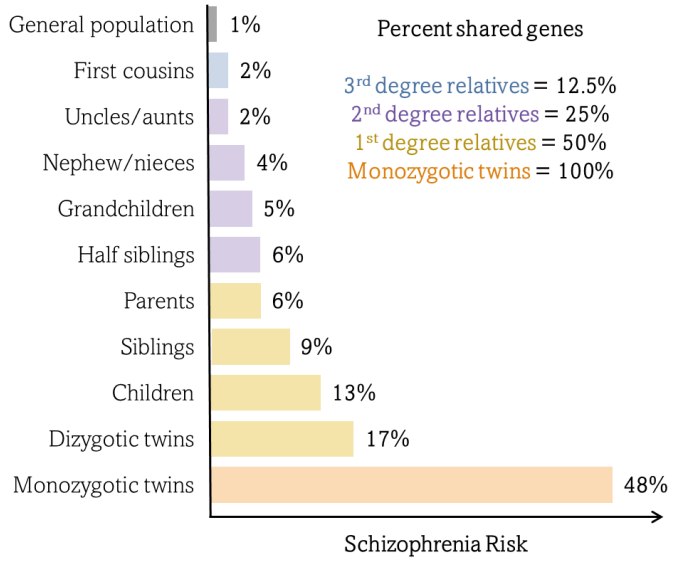


Figure 6. Rates of schizophrenia among relatives of patients with schizophrenia. The figure depicts the increasing risk of developing schizophrenia depending on the increasing number of shared genes between family members and their affected relatives. Adapted figure (Gottesman, 1991).

However, while familial clustering strongly suggests a genetic contribution, family studies alone cannot distinguish whether this is due to genetic factors or shared familial

environmental influences. In this regard, adoption studies help to distinguish genetic and environmental influences on family resemblance by comparing rates of the disorder in biological family members, that are genetically related, to those in adoptive families, that are environmentally related. These studies have indicated that adoptees with biological progenitors affected by schizophrenia, which are supposed to carry a stronger genetic liability, not only present a higher risk towards schizophrenia-spectrum disorders but also are more sensitive to environmental problems in the adoptive family (Tienari et al., 2004).

Despite this body of evidence highlighting the significant role of genetics, it does not allow for an exact estimation of their contribution. In this context, twin studies, conducted to estimate the genetic and environment contributions to the variance in liability to the disorder, have shown that schizophrenia is much more likely to occur in both members of monozygotic (MZ) twin pairs, with concordance rates between 41% and 65%, compared to 0% to 28% in dizygotic (DZ) twins (Cardno & Gottesman, 2000; Gottesman, 1991). Since both types of twins are raised in the same environment but differ in the amount of genes they share, with MZ twins sharing 100% and DZ twins 50%, the higher concordance in MZ twins suggests that familial aggregation of schizophrenia is mainly due to genetic factors. Moreover, the observed similar risk of developing schizophrenia in the children of unaffected and affected MZ twins, compared to the higher risk in children of affected DZ twins, suggests that unaffected MZ twins may carry unexpressed susceptibility genes for the disorder (Gottesman & Bertelsen, 1989). However, more importantly, these approaches have enabled researchers to estimate the proportion of phenotypic variability due to genetic factors, known as heritability (h^2), which is currently estimated to be between 60-80%, underscoring the significant role of genetics (Hilker et al., 2018; Sullivan et al., 2003).

Given the prominent role of genetics in the etiology of schizophrenia, vast research has focused on unraveling its genetic architecture to better understand its pathophysiology and improve diagnosis and treatment strategies. Molecular studies have revealed that schizophrenia is highly polygenic, with genetic risk stemming from the cumulative effects of thousands of common alleles, such as single nucleotide polymorphisms (SNPs) and copy number polymorphisms (CNPs), and few rare mutations with greater impact, like single nucleotide variants (SNVs) and copy number variants (CNVs) (Foley et al., 2017; Legge, Santoro, et al., 2021; Owen et al., 2023) (**Box 3**).

In the search for genes and specific variants linked to schizophrenia risk, hypothesis-driven research has employed two main approaches focused on candidate gene studies. This include methodologies such as case-control comparisons, where genetic variant frequencies are contrasted between affected individuals and healthy controls, and family-based approaches, which examine the transmission of specific alleles from heterozygous parents to affected offspring compared to the non-transmitted alleles (Ewens & Spielman, 1995). Studies performed with families have the advantage of reducing population heterogeneity by focusing on genetically similar pedigrees with consistent environmental

exposures (Glahn et al., 2019). However, collecting sufficiently large and well-characterized family samples can be challenging, making case-control studies more scalable. In these studies, genes are often selected based on positional evidence from linkage studies or structural variations (e.g., *COMT*, *DAO*, *DISC1*, *DTNBP1*, *NOTCH4*, *NGR1*), or based on hypotheses regarding the etiology of schizophrenia, such as the dopaminergic hypothesis (e.g., *DRD1*, *DRD3*, *DRD4*, *COMT*), or due to pharmacological relevance (e.g., *HTR2A*, *SLC6A3*, *SLC6A4*) (Collins et al., 2012). These studies have made significant contributions to our understanding of schizophrenia etiology, with over 1,700 genetic association studies cataloged, covering 1,008 genes and 8,788 polymorphisms (Allen et al., 2008). Moreover, meta-analytic results have further reinforced the role of these candidate genes in schizophrenia (Glatt et al., 2003; González-Castro et al., 2016; Jagannath et al., 2018; Li et al., 2016; Mohamed et al., 2018; Watanabe et al., 2013).

Complementing the approaches mentioned, genome-wide association studies (GWAS) employ a hypothesis-free method to analyze millions of common genetic variants across the genome, comparing their frequencies between individuals with schizophrenia and controls. This method has proven more effective than candidate gene studies in identifying genetic variants associated with schizophrenia. However, due to the vast number of variants analyzed, GWAS requires large sample sizes and stringent significance thresholds (typically $p < 5 \times 10^{-8}$) to minimize false positives. Larger samples increase statistical power, enabling the accurate identification of independent loci. For example, the four GWAS conducted by the Psychiatric Genomics Consortium have progressively expanded in size and thus in the identified variants, with the latest study identifying 287 loci across a sample of 76,755 patients and 243,649 controls (Pardiñas et al., 2018; Ripke et al., 2014; Trubetskoy et al., 2022).

Importantly, GWAS objectives extend beyond identifying specific genes. Pathway and gene set enrichment analyses have shown that the identified genetic variants converge on biological mechanisms like those highlighted in earlier candidate gene studies. Specifically, the latest GWAS reports that schizophrenia-associated genes are primarily expressed in excitatory and inhibitory neurons of the central nervous system. These genes also converge into biological processes related to development, neuronal differentiation, neuronal function, and synaptic transmission and involve key cellular components like ion channels and synapses (Trubetskoy et al., 2022).

Additionally, these studies have introduced a method to quantify the genetic burden each individual carries for a given trait, known as the polygenic risk score (PRS). This is a valuable tool for estimating the cumulative genomic risk of a person, calculated by summing the additive effects of each genotyped variant and weighting them according to effect sizes estimated in genome-wide association studies (Choi et al., 2020). While the PRS for schizophrenia is not intended as a diagnostic tool, it serves as a highly informative measure for assessing individual risk for the disorder in research contexts and has shown strong consistency across studies and sample groups (Raben et al., 2022). A higher

schizophrenia PRS has been observed not only in schizophrenia patients but also in high-risk individuals and first-degree relatives (Perkins et al., 2020; van Os et al., 2020).

To expand the search for genetic contributors to schizophrenia, researchers have also focused on identifying rare genetic variants. However, this effort has proven challenging due to the polygenic nature of schizophrenia, the scarcity of harmful variants subject to natural selection, and the vast presence of benign rare variations within the population. Early methodologies such as fluorescence in situ hybridization (FISH) or comparative genomic hybridization (CGH) identified the 1.5–3 MB deletion in 22q11.2, with individuals carrying this deletion have a lifetime risk of developing schizophrenia of approximately 25–30%, significantly higher than the general population risk of around 1% (Van et al., 2017). With the advent of genome technologies, such arrays and whole-exome or whole-genome sequencing, more rare variants have been identified. The latest and biggest whole-genome array study, involving over 40,000 cases and controls, has identified eight CNVs associated with schizophrenia risk, with *NRXN1* exonic deletions being the only CNV linked to gene disruption. Although these deletions are rare in general population, they affect wide range of cases, spanning from 0.015%–0.64%, and confer a notably high risk for schizophrenia, with odds ratios ranging from 1.8 to 81.2 (Rees & Kirov, 2021). Additionally, individuals with schizophrenia show an enrichment of CNVs larger than 20 KB compared to controls, particularly those overlapping genes, which carry the strongest risk effects (Marshall et al., 2017). Meanwhile, exome-sequencing studies focusing on SNVs and indels, particularly in parent-offspring trios, have shown that de novo damaging coding variants in schizophrenia cluster in genes intolerant to protein-truncating variants (PTVs), genes implicated in early-onset neurodevelopmental disorders, and associated with glutamatergic postsynaptic proteins (Fromer et al., 2014; Howrigan et al., 2020). Also, case-control exome-sequencing studies have revealed an enrichment of ultra-rare damaging coding variants within these same gene sets in schizophrenia patients (Genovese et al., 2016; Singh et al., 2022).

Importantly, recent GWAS estimates that SNP-based heritability accounts for around 24% of the schizophrenia risk (Trubetskoy et al., 2022). In comparison, the heritability attributed to SNVs and indels is approximately 2%, which is also the estimated contribution of CNVs (Weiner et al., 2023). This still leaves the issue of missing heritability, the gap between the variance explained by common variants and the higher estimates from twin studies. This difference arises because the genetic architecture of schizophrenia involves both interactions between genes (epistasis) and gene-environment interactions (Zuk et al., 2012). However, deciphering the epistatic networks involved in schizophrenia presents a significant methodological challenge. While meta-analyses using pairwise testing of independent loci have not yielded success, a more recent study employing discrete discriminant analysis, which examines a broader range of interacting partners beyond SNP pairs, demonstrated that collective interaction analysis significantly enhanced the association with schizophrenia (Ripke et al., 2014; Woo et al., 2017). Pathway analysis of these SNP networks suggested a disease mechanism in which

maternal immune activation disrupts neurogenesis and causes deficits in cortical interneurons, ultimately leading to symptoms triggered by synaptic pruning (Woo et al., 2017).

Box 3. Human genome and genetic variants

The diploid human nuclear genome, which contains the complete set of DNA instructions in each cell, is composed of approximately 3.2 billion nucleotides in males and 6.4 billion in females. These nucleotides, known as adenine, cytosine, guanine, and thymine, are arranged across 24 chromosomes, including 22 autosomes and two sex chromosomes, X and Y. The specific sequence of these nucleotides forms the genetic blueprint that guides the body's development and function. The human genome includes around 60,498 predicted genes. Of these, 19,881 are responsible for coding proteins, while 25,813 are non-coding, and the rest are considered pseudogenes. Interestingly, the information for protein-coding genes is found within only 1.5% of the entire genome (Brown, 2002).

Approximately 0.4% of the nucleotides in the human genome vary between individuals, contributing to genetic diversity and differing disease susceptibility. These genomic variants include single-nucleotide variants (SNVs), where a single nucleotide is altered, and indels, involving the insertion or deletion of fewer than 50 nucleotides. Copy number polymorphisms (CNPs) are short, repeated sequences that vary among individuals. Larger genomic variations stem from structural changes, where regions of 50 to thousands of nucleotides are inserted, deleted, inverted, relocated, or repeated, known as copy-number variants (CNVs) (Bachtiar et al., 2019). The 1000 Genomes Project revealed a wide range of genetic diversity, including 84.7 million SNVs, 3.6 million indels, and 60,000 structural variants. On average, an individual's genome contains about 5 million SNVs, 600,000 indels affecting 2 million nucleotides, and 25,000 structural variants spanning over 20 million nucleotides compared to a reference genome (Auton et al., 2015).

The genetic makeup of an individual is heavily influenced by their ancestry, with allele frequencies within a population varying according to ethnic background. Given the population rate of the less frequent variant (allele), also referred to as minor allele frequency (MAF), the genetic variants are classified as common (MAF >1%), uncommon (MAF 0.1-1%), rare (MAF <0.1%), and ultra-rare (MAF <0.001%). Different types of genomic variants with varying MAFs contribute distinctly to psychotic disorders, as illustrated in the figure below (Sullivan et al., 2024).

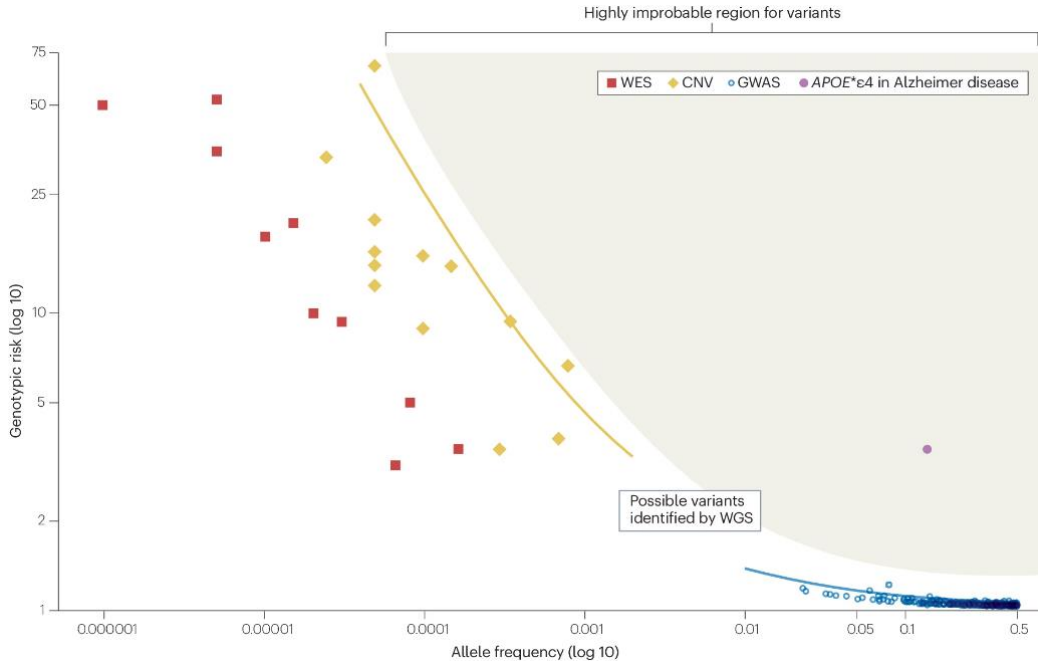


Figure B3. Frequency of genetic variants in healthy population and their size effect in relation to schizophrenia risk. The figure shows that rare variants found through whole-exome sequencing (WES) and CNVs (on the left side) are rare but have strong effects, while those common variants discovered via GWAS (on the right) are common but have weaker effects. Adapted figure (Sullivan et al., 2024).

Epigenetics

Adding further complexity to the etiology of schizophrenia epigenetic mechanisms are also involved, as well as transcriptional and post-transcriptional regulation. These processes contribute to cellular diversity in function and structure despite the uniformity of the genetic code (Franks et al., 2017). Key regulatory levels include control of gene expression via methylation and histone modifications, which influence chromatin accessibility, alongside processes that regulate RNA splicing, processing, and degradation (Bacon & Brinton, 2021; Föcking et al., 2019; Reichard & Zimmer-Bensch, 2021; Richetto & Meyer, 2021; Smigielski et al., 2020).

Prominently studied among these mechanisms is DNA methylation (Moore et al., 2013) (Box 4). However, the relationship between methylation and gene expression is believed to be complex, with the balance between methylation levels in the promoter and gene body regions being crucial for effective regulation (Wagner et al., 2014). In the brain, methylation plays a twofold role: during development, the balance between methylation of germ line-specific genes that suppress pluripotency and demethylation of neuron-specific genes is key to facilitate neuronal specialization; in the mature brain, methylation changes can be induced by neuronal activity, contributing to complex cognitive processes such as learning and memory (Halder et al., 2016; Murao et al., 2016; Sun et al., 2016). The crucial nature of these mechanisms is evident in mice lacking methylation machinery, which display abnormal synaptic plasticity and cognitive deficits (Feng et al., 2010). Additionally, it is well-described that methylation processes are influenced by environmental stimuli (Jirtle & Skinner, 2007), leading to changes, some of which may have lasting effects, thus directly impacting gene expression and shaping the phenotype (Jaenisch & Bird, 2003; Perera et al., 2020).

Much of the methylation research has focused on peripheral tissues, though this comes with the limitation of not directly addressing brain-specific processes. Interestingly, methylation differences have been observed in monozygotic twins discordant for schizophrenia (Dempster et al., 2011; Fisher et al., 2015), and similar changes have been detected in individuals at ultra-high risk for the disorder (Ciuculete et al., 2017). Notably, specific methylomic alterations have also been reported to emerge during the transition to psychosis (Kebir et al., 2017). Additionally, several methylome-wide association studies have identified significant differences between schizophrenia patients and healthy controls (Aberg et al., 2014; Hannon et al., 2016; Montano et al., 2016). A meta-analysis has further highlighted that many of these studies converge on a significant cluster of genes linked to synaptic membrane function and structure, as well as genes involved in immune and stress responses (Chan et al., 2020). Although peripheral methylation may not capture all brain-related disease changes, some studies have shown promise by revealing significant correlations in methylation between blood and brain tissue (Davies et al., 2012; Walton et al., 2016). Notwithstanding, beyond these tissue correlations, investigating blood methylation remains valuable for biomarker identification, particularly given the accessibility of the tissue and its reversible nature,

which enables the examination of therapy-induced changes potentially leading to enhanced patient care (Goud Alladi et al., 2018).

Box 4. DNA methylation mechanism

The process of DNA methylation consists of the covalent addition of a methyl group to cytosines to form 5-methylcytosine (5-mC) (Moore et al., 2013). This process is catalyzed by DNA methyltransferase proteins (DNMTs) that use S-adenosyl-methionine (SAM) as a primary methyl group donor and that is converted to S-adenosine homocysteine (SAH) during the process. Three conserved DNMT enzymes are responsible for the methyl mark: DNMT3A and DNMT3B catalyze de novo DNA methylation, whereas DNMT1 represents the maintenance enzyme, which restores the fully methylated state of DNA after replication (Feng et al., 2010). The majority of 5-methylcytosine is frequently located in clusters of CpG dinucleotides, called CpG islands, which are frequently found in gene promoter regions. This mark is generally a repressive mark since it acts by blocking gene transcription, but this depends on the genomic context (Katirtzoglou et al., 2024).

Schematic representation of the methylation process

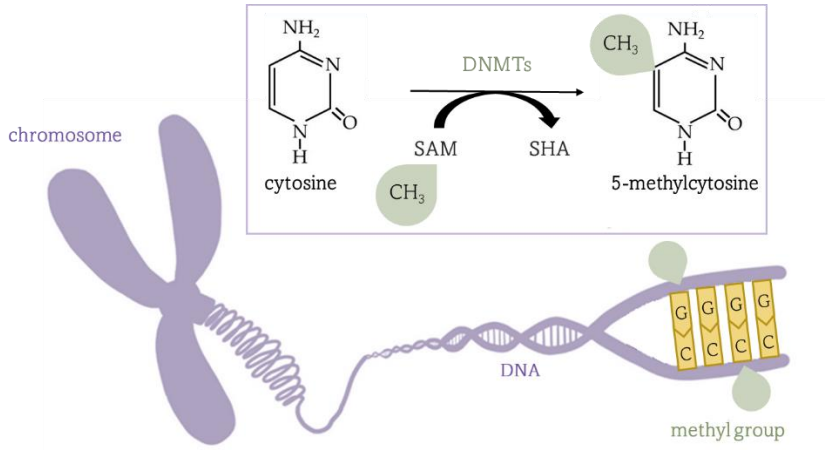


Figure B4. Schematic representation of DNA methylation process. The figure shows, at the top, the reaction through which DNA methyltransferase proteins (DNMTs) add methyl groups using S-adenosyl-methionine (SAM) as a primary methyl group donor and converted to S-adenosine homocysteine (SAH) during the process transforming cytosine nucleotides into 5-methylcytosine. Adapted figure (Health, 700).

Methylation research has also been conducted using post-mortem brain tissue, as obtaining living brain tissue through biopsies in schizophrenia patients is exceedingly rare. Studies using human prefrontal cortex or hippocampus tissues have revealed important differences in several well-established schizophrenia risk genes, including *RELN*, *DRD2*, *COMT*, and HTR2A (Dempster et al., 2011; Ghadirivasfi et al., 2011; Guidotti et al., 2016; A. Zhang et al., 2007). Moreover, whole methylation studies have unveiled novel genes implicated in neuron development, synaptogenesis, synaptic transmission, and plasticity (Alelú-Paz et al., 2016; Chen et al., 2014; McKinney et al., 2017; Oord et al., 2015; Pidsley et al., 2014; Ruzicka et al., 2015, 2017; Viana et al., 2016; Wockner et al., 2014; Zhao et al., 2015).

Simultaneously, other studies have examined gene expression, initially focusing on candidate genes through PCR analysis, though primarily using microarrays and RNA sequencing, to understand how genetic and environmental factors interact in the modulation of schizophrenia (Wu et al., 2017). Much like methylation research, most gene expression studies have focused on peripheral tissues, with a recent review identifying dysregulated proline metabolism genes in blood. Additionally, this study suggests a limited but notable overlap among genetic, epigenetic, and gene expression changes, underscoring the interplay between genetic and environmental factors in schizophrenia (Wagh et al., 2021). But these type of studies have been also performed in post-mortem brain tissue, specially using prefrontal cortex or hippocampus, with a recent review emphasizing the importance of genes related to circadian rhythms, immune response and inflammation, alongside mitochondrial energy metabolism and oxidative phosphorylation, myeloid leukocyte activation, cytoskeletal proteins, ion transport regulation, neural development, neurite outgrowth, and synaptic plasticity (Merikangas et al., 2022).

Moreover, considering the impact of methylation on gene expression, numerous studies have investigated their relationship in post-mortem brain samples from individuals with schizophrenia. These studies found that hypomethylation of the promoter regions of most candidate genes associated with dopaminergic (*DRD2*, *DRD3*, *COMT*), GABAergic (*GAD1*), serotonergic (*HTR2A*), oligodendrocyte (*SOX10*), and synaptic plasticity (*RELN*) functions correlate with increased expression of these genes in the brain in SZ (Huang & Akbarian, 2007; Iwamoto et al., 2005; Kordi-Tamandani et al., 2013; Kundakovic et al., 2007). In parallel, multi-omics studies integrating transcriptomics and genome-wide methylation have confirmed this relationship for the classical candidate genes and uncovered novel correlations in other genes involved in nervous system development, adaptive immune response, and neuronal metabolism (Chen et al., 2014). Interestingly, these studies examining both methylation and gene expression, suggest that these differences may be specific to particular brain regions and cell types, with distinct genes involved in each context (Collado-Torres et al., 2019).

Notably, all the evidence derived from the research investigating etiological factors in schizophrenia align with the neurodevelopmental hypothesis, which proposes that genetic risk variants disrupt brain development and synaptic plasticity, ultimately impairing the refinement of synapses and formation of functional neural circuits, leading to a broad spectrum of clinical and subclinical symptoms later in life.

1.7. Neuritin-1 as a model for unraveling the path from genes to clinical outcomes

Despite progress in psychiatric genetics, significant gaps remain in linking genetic variants to schizophrenia phenotypic diversity. Whole-genome studies, while successful, often overlook variability within cases due to their focus on large, homogeneously diagnosed samples. To better understand how genetic variants influence clinical outcomes, candidate gene studies typically follow up on these findings in more deeply phenotyped cohorts. Given the wide range of symptoms in psychotic disorders, likely due to genetic diversity, addressing clinical heterogeneity is crucial for improving the accuracy of genetic association analyses in schizophrenia. Nonetheless, as mentioned through the introduction section, the heterogeneity of schizophrenia manifests across multiple levels, ranging from genetic and expression differences to the involvement of distinct cell types, as well as variations in brain structure and function, all of which contribute to variability in cognition, symptoms, treatment responses, and ultimately diagnosis.

Accordingly, this thesis employed a whole-genome-derived candidate gene approach, with a particular focus on *NRN1*, to elucidate the genotype-phenotype relationship in schizophrenia. Using a multilevel strategy, we aimed to demonstrate how plasticity genes influence key schizophrenia-related phenotypes, such as age at onset, clinical and functional traits, cognitive performance, and neuroimaging measures, specifically highlighting the role of *NRN1*. This approach aims to illustrate an effective method for identifying the specific mechanisms by which genes contribute to the biological processes underlying schizophrenia.

The role of Neuritin-1 in brain health and disease

Neuritin-1 (*NRN1*), also known as Candidate Plasticity Gene 15 (*CPG15*), was first identified in a screen for genes regulated by neuronal activity that are involved in synaptic plasticity (Nedivi et al., 1996). Located on chromosome 6p25.1, approximately 6 Mb from the telomere, *NRN1* encodes a small protein of 142 amino acids with a molecular mass of 15.3 kDa. The protein features a hydrophobic N-terminal region (amino acids 1–27) that acts as a signal peptide for secretion, while its C-terminal tail, rich in hydrophobic residues, contains a consensus cleavage site characteristic of GPI-anchored proteins (amino acids 116–142). The orthologous *NRN1* gene in rats (*Nrn1*) is highly conserved, sharing 98% sequence identity with the human gene, differing by only 14 base pairs (Naeve et al., 1997).

From animal-based assays, it has been demonstrated that during early embryonic development, *Nrn1* expression acts as a survival factor for neural progenitors (Putz et al., 2005). As development progresses, *Nrn1* plays a pivotal role in supporting neuronal migration (Zito et al., 2014), promoting dendritic and axonal outgrowth, and facilitating synaptic maturation during neuronal differentiation (Cantallops et al., 2000; Cappelletti

et al., 2007; Corriveau et al., 1999; Fujino et al., 2008, 2011; Javaherian & Cline, 2005; Lee et al., 2005; Naeve et al., 1997; Nedivi et al., 1998, 2001; Sato et al., 2012). After birth, *Nnr1* expression continues to be elevated in the adult brain, especially in areas with high neural activity and synaptic plasticity, such as the visual cortex, the hippocampus, or the external granular layer of the cerebellum, being expressed in an activity-dependent manner (Chen et al., 2011; Fujino et al., 2003; Harwell et al., 2005; Lee & Nedivi, 2002; Naeve et al., 1997; Nedivi et al., 2001; Putz et al., 2005; Tiruchinapalli et al., 2008).

At the cellular level, Nrn1 is trafficked to and from the axonal surface in response to membrane depolarization, localizing to vesicles and endosomes alongside other synaptic vesicle proteins (Cantallops et al., 2000; Nedivi et al., 2001).

Particularly, the *NRN1* gene is classified as an immediate early gene, meaning its expression is rapidly activated by specific pathways in response to regulatory signals. As a result, *Nrn1* expression is not only influenced by synaptic activity and sensory experience but is also highly sensitive to a variety of stimuli in both physiological and pathological conditions, including hormones like testosterone, gonadal steroids, and androgens, as well as seizures and neurotrophic factors such as *NGF*, *BDNF*, and *NT3* (El-Sayed et al., 2011; Marron et al., 2005; Tetzlaff et al., 2006).

Notably, the transcriptional activation of *NRN1* is a crucial link between neuronal activity and long-term synaptic plasticity. As an immediate early gene, *Nrn1* expression is stimulated by calcium (Ca^{2+}), a key player in neuronal signaling. Calcium enters neurons through channels such as N-methyl-D-aspartate (NMDA) receptors and L-type voltage-gated calcium channels (VGCCs). Once inside, the calcium influx activates critical signaling pathways, including the Ca^{2+}/calmodulin-dependent protein kinase (CaMK) pathway and the mitogen-activated protein kinase (MAPK) pathway. These pathways regulate various cellular processes, particularly the expression of plasticity-related genes like *NRN1*, ultimately leading to AMPA recruitment and long-term changes in synaptic strength and plasticity, which are essential for learning and memory (Fujino et al., 2003). Then, it seems that *NRN1* is playing a role in the dysfunction in glutamate signaling, that activates both AMPA and NMDA.

Likewise, biochemical analyses reveal that NRN1, as a soluble molecule, can activate the insulin receptor (IR), though another study suggests it may also bind to the insulin-like growth factor type 1 receptor (IGF-1R). Regardless, NRN1 activates critical pathways, including MEK-ERK, PI3K-Akt-mTOR, and Ca2+-CaN-NFATc4, leading to increased transient outward potassium currents and Kv4.2 expression, as well as the upregulation of CaV1.2 and CaV1.3 subunits of L-type voltage-gated calcium channels (VGCCs) in the cell membrane of cerebellar granule neurons (Yao et al., 2012; Zhao et al., 2018). Together, these effects support processes essential for learning and memory. However, NRN1 is still considered as an orphan ligand and its receptor remains unknown.

Interestingly, there is compelling evidence linking *NRN1* to psychiatric disorders. Increased *Nrn1* expression is linked to enhanced cognitive performance in mice, including improved spatial learning, memory recovery after ischemia-reperfusion injury, better neurological scores after traumatic brain injury, and reduced cognitive impairments in Alzheimer's models, while its decrease expression is associated with depression (An et al., 2014; Liu et al., 2018; Reshetnikov et al., 2020; Son et al., 2012; Wan et al., 2020). Also, *Nrn1* responds to neurotherapeutic agents, such as electroconvulsive therapy and fluoxetine, which alter its expression through epigenetic pathways involving histone deacetylations (Alme et al., 2007; Dyrvig et al., 2014; Newton et al., 2003; H. G. Park et al., 2014). Moreover, human-based studies have implicated the *NRN1* gene in the risk for mental disorders, as well as in early onset and cognitive deficits in patients, highlighting its clinical relevance (Chandler et al., 2010; Fatjó-Vilas et al., 2016).

In summary, the temporal expression of the *NRN1* gene plays a key role in regulating neuronal numbers by promoting progenitor expansion, preventing apoptosis in specific neuroblast subpopulations, and ensuring proper circuit maturation, ultimately influencing brain structure and function. Furthermore, *NRN1* continues to be expressed in the adult brain, where it contributes to synaptic plasticity, enabling the brain to adapt to internal and external stimuli. Importantly, animal studies have shown that *Nrn1* expression can be modulated, with significant effects on behavioral phenotypes. Additionally, its polymorphic variability has been linked to an increased risk of schizophrenia spectrum disorders and related clinical phenotypes. In this context, developing strategies to target *NRN1* or its associated signaling pathways could offer a promising approach for improving schizophrenia treatment, particularly in addressing cognitive symptoms that do not respond to traditional antipsychotic therapies.

Reducing schizophrenia clinical heterogeneity

Using specific phenotypes with clear etiological significance, such as age at onset, is an effective strategy for reducing heterogeneity and identifying genetic factors associated with schizophrenia. Family studies reveal strong correlations in age at onset between probands and their relatives, with proband-sibling correlations ranging from 0.6 to 0.9 (Crow & Done, 1986). Twin studies further highlight the genetic contribution, showing a 4.7-fold higher familial risk in early-onset schizophrenia twin pairs (before age 22) compared to late-onset cases, along with closer age at onset in monozygotic twins compared to dizygotic twins (Hilker et al., 2017). These approaches have also underscored the important role of genetic factors in determining age at onset, with heritability estimates around 33% (Hare et al., 2010). Unraveling the genetic basis of age at onset is of significant clinical importance, as early-onset schizophrenia is linked to stronger familial aggregation of schizophrenia and other psychiatric disorders, poorer premorbid adjustment and neurocognitive functioning, more severe symptoms, and worse outcomes with a poorer prognosis (Baykal et al., 2024; Payá et al., 2013). These

findings indicate that early-onset cases are associated with more pronounced neurodevelopmental disruptions compared to adult-onset forms. This approach has identified several candidate genes, such as *DRD2*, *DRD3*, *COMT*, *BDNF*, and *NRG1*, as potential modulators of age at onset in schizophrenia, through correlations with quantitative measures or by distinguishing early from adult-onset cases (Alfimova et al., 2023; Mané et al., 2017; Voisey et al., 2012; Yoshimi et al., 2016). In the context of GWAS analysis, while there has been a significant increase in research across various domains, few studies have focused on age at onset (Bergen et al., 2014; Guo et al., 2021; Wang et al., 2011; Woolston et al., 2017). Interestingly, the variants linked to an earlier age at onset in these studies converge within molecular networks associated with nervous system development, axon extension regulation, glial proliferation, molecular transport, and cell-to-cell signaling. However, replication at the individual SNP level remains limited across studies, likely due to differences in defining age at onset. Additionally, early-onset patients exhibit higher schizophrenia PRS compared to their siblings, with these scores effectively predicting an earlier onset of symptoms (Ahn et al., 2016).

Another approach to reducing heterogeneity is to examine the impact of genetic variability on symptom severity. Symptoms in schizophrenia are well-defined and operationalized, with validated and reliable tools available for their assessment. Research has shown that symptom dimensions are not only familial but also heritable (Malaspina et al., 2000). Several candidate gene studies have successfully linked genetic variants to specific symptom dimensions (Fanous et al., 2005; Nieto et al., 2021; Yang et al., 2021; Zahari et al., 2011). However, whole-genome studies suggest that schizophrenia is not a single disorder but rather a group of heritable conditions caused by distinct genotypic networks, each associated with particular symptom profiles (Arnedo et al., 2015). Additionally, studies using PRS or genetic risk scores (GRS) for schizophrenia have demonstrated correlations with measures of functioning (e.g., GAF) and general psychopathology (e.g., PANSS), indicating that each dimension is partly influenced by separate genetic factors (Edwards et al., 2016; Santoro et al., 2018; Sengupta et al., 2017).

Additionally, neuroimaging data offers valuable insights into the contribution of genetic factors to the neurobiology of the disorder. Heritability estimates for traits such as brain functional response range from 40 to 65%, while for cortical thickness and surface area estimates are between 52 and 78%, underscoring a substantial genetic influence on their variability (Jansen et al., 2015). Additionally, numerous neuroimaging genetic studies have demonstrated the specific impact of schizophrenia risk genes, such as *BDNF*, *CACNA1C*, *COMT*, *ZNF804A* and *DRD2*, on brain structure and function (Jiang et al., 2019). Moreover, several studies have linked a higher schizophrenia PRS to reduce cortical thickness alterations in high-risk individuals, patients with schizophrenia, and even healthy individuals, and also to cognitive abilities, such as working memory and processing speed, and consequently to altered brain activity during these tasks (Benca et al., 2016; Cattarinussi et al., 2022; He et al., 2021; Lencz et al., 2013; Miller et al., 2018; Ohi et al., 2021).

2. Hypothesis & Objectives

Hypothesis

Given the significant genetic component of schizophrenia, understanding the role of genetic factors could provide valuable insights into the biology of the disease. This understanding may ultimately support the development of novel therapies and better treatment strategies that will improve patients' quality of life.

Evidence increasingly shows that synaptic plasticity, regulated by neuronal activity, plays a crucial role in brain function throughout development and adulthood. In schizophrenia, this process is disrupted, with many associated genes converging on related pathways. Despite these advances, there are still considerable gaps in connecting genetic variants to the phenotypic diversity observed in schizophrenia. Much of this difficulty arises from the extensive biological heterogeneity that characterizes the disorder.

This heterogeneity is evident not only in clinical presentations but also across various levels, from genetic variation to diagnostic classifications. Building on this rationale, we hypothesized that a multilevel approach incorporating various aspects of *NRN1* molecular diversity, including the association of its genetic variants with age at onset, clinical and neuroimaging traits, its interaction with related genes, its expression and methylation patterns in postmortem brain samples from schizophrenia patients, and its methylation changes in response to cognitive therapy, could provide a comprehensive understanding of how genes influence the biological processes underlying schizophrenia.

The hypotheses of the present thesis are:

HYPOTHESIS I: *NRN1* genetic variability will be associated with an increased risk of schizophrenia, particularly in early-onset forms of the disorder. The neurobiological basis of this association will be evidenced by the role of *NRN1* gene in the functional and structural brain differences observed in patients compared to healthy subjects and also by its modulation of clinical manifestations. In addition, these effects will be further shaped by epistatic effects between *NRN1* and other molecularly related genes, such as *BDNF* and *CACNA1C*.

HYPOTHESIS II: *NRN1* methylation and expression levels in the brain will be correlated and will differ between individuals with schizophrenia and control subjects. These differences will be modulated by antipsychotic treatment and influenced by genetic variability within the locus.

HYPOTHESIS III: *NRN1* peripheral epigenetic changes following Cognitive Remediation Therapy (CRT) will be associated with cognitive improvements, with this effect further influenced by genetic variability within the locus.

Objectives

The hypotheses were explored through aligned objectives, organized into sections:

Section I: The role of NRN1 genetic variability in schizophrenia risk, clinical expression, intermediate phenotypes, and its interactions with related genes

I.I. To investigate the association of *NRN1* gene with the age at onset of schizophrenia, through the analysis of 11 single nucleotide polymorphisms (SNPs) and using two complementary approaches: family-based and case–control, including in both, and early-onset (EO) versus adult-onset (AO) cases of the disorder. Additionally, to investigate whether the genetic variants associated with EO differentially impact working memory-related brain activity, as assessed through the N-back neuroimaging protocol.

I.II. To assess the epistatic effect of polymorphisms in the *NRN1* gene, measured by 11 SNPs, along with *BDNF* (rs6265) and *CACNA1C* (rs1006737), on schizophrenia risk, age at onset, clinical severity and functionality. Next, to explore the joint influence of these polymorphisms on neuroanatomical cortical measures, such as thickness, surface area, and volume. Finally, to investigate whether brain regions under significant epistatic effects mediate the clinical outcomes in patients.

Section II: Brain NRN1 genetic, epigenetic, and expression correlates and the modulatory effect of antipsychotic treatment

II. To investigate whether *NRN1* mRNA expression and methylation profiles across three CpG islands in brain post-mortem samples of the prefrontal cortex and hippocampus differ between control subjects and schizophrenia patients; with particular focus on the influence of the antipsychotic treatment by comparing clozapine use to no antipsychotic medication at the time of death. Additionally, to explore whether genetic variability, measured by 11 SNPs, influences this correlation.

Section III: The impact of NRN1 genetic variability and peripheral epigenetic changes on cognitive improvements following Cognitive Remediation Therapy (CRT)

III. To assess whether *NRN1* methylation changes across three CpG islands in blood peripheral samples differ between patients undergoing Cognitive Remediation Therapy (CRT) and those receiving treatment as usual (TAU). Additionally, to explore whether these methylation changes are linked to cognitive improvement, considering the influence of genetic variability, measured by 11 SNPs, on this relationship.

3. Supervisor's Report

The doctoral thesis "*The role of the synaptic plasticity gene NRN1 in schizophrenia: integrating molecular and neuroimaging approaches*" presented by Carmen Almodóvar Payá is based on the original results obtained by the doctoral candidate.

These results are centred on the assessment of the genetic influence of *NRN1* gene, a synaptic plasticity gene, on the risk for schizophrenia and different clinical and neurobiological phenotypes related to the disorder. This has been conducted through different research designs and methodological approaches that have allowed to add new data on the impact of genetic, epigenetic and expression variability of *NRN1* in the origin and manifestation of the disorder, as well as in treatment response.

Four original research articles are presented: two have been published, one is accepted, and one submitted, all in international peer-reviewed journals.

Study I.I. *NRN1* Gene as a Potential Marker of Early-Onset Schizophrenia: Evidence from Genetic and Neuroimaging Approaches

Almodóvar-Payá C, Guardiola-Ripoll M, Giralt-López M, Gallego C, Salgado-Pineda P, Miret S, Salvador R, Muñoz MJ, Lázaro L, Guerrero-Pedraza A, Parellada M, Carrión MI, Cuesta MJ, Maristany T, Sarró S, Fañanás L, Callado LF, Arias B, Pomarol-Clotet E, Fatjó-Vilas M. International Journal of Molecular Sciences. 2022 Jul 5;23(13):7456. doi: 10.3390/ijms23137456. PMID: 35806464

The International Journal of Molecular Sciences is an international peer-reviewed journal providing an advanced forum for molecular and cell biology, molecular medicine, and all aspects of the molecular research.

According to the Journal Citation Reports (Science Edition, 2023), the impact factor of the journal is 4.9 and it is ranked in the 79.1% percentile of the area of Biochemistry & Molecular Biology (66/313), which corresponds to the first quartile (Q1).

The doctoral candidate has participated in the study conception, data curation, the statistical analysis, interpretation of the results and the visual presentation of the findings. As well, the doctoral student wrote the first version of the manuscript and conducted the revision and edition of the subsequent draft versions.

Study I. II. *NRN1* epistasis with *BDNF* and *CACNA1C*: mediation effects on symptom severity through neuroanatomical changes in schizophrenia

Almodóvar-Payá C, Guardiola-Ripoll M, Giralt-López M, Oscoz-Irurozqui M, Canales-Rodríguez EJ, Madre M, Soler-Vidal J, Ramiro N, Callado LF, Arias B, Gallego C, Pomarol-Clotet E, Fatjó-Vilas M. Brain Structure and Function. 2024 Jun;229(5):1299-1315. doi: 10.1007/s00429-024-02793-5. PMID: 38720004

The Brain Structure and Function is an international peer-reviewed journal that publishes research that provides insight into brain structure–function

relationships by integrating data spanning from molecular, cellular, developmental, and systems architecture to the neuroanatomy of behavior and cognitive functions.

According to the Journal Citation Reports (Science Edition, 2023), the impact factor of the journal is 2.7 and it is ranked in the percentile 93.1% of the area of Anatomy & Morphology (2/22), which corresponds to the first decile (D1).

The doctoral candidate participated in the study conception, DNA extraction, and genotyping. Also, the candidate had a prominent role in the data curation, the statistical analysis, the interpretation and the visual presentation of the findings. As well, the doctoral student wrote the first version of the manuscript and conducted the revision and edition of the subsequent versions.

Study II. Clozapine-related brain *NRN1* expression patterns are associated with methylation and genetic variants in schizophrenia.

Almodóvar-Payá C, Moreno M, Guardiola-Ripoll M, Latorre-Guardia M, Morentin B, Garcia-Ruíz B, Pomarol-Clotet E, Callado LF, Gallego C, Fatjó-Vilas M. Submitted for peer review in an indexed journal.

The doctoral candidate participated in the study conception, sample management, DNA extraction, genotyping-methylation-expression data processing and curation, methodology and statistical analysis, the interpretation and the visual presentation of the findings. Also, the doctoral student wrote the first version of the draft and conducted the revision and edition of the subsequent versions.

Study III. *NRN1* genetic variability and methylation changes as biomarkers for cognitive remediation therapy response in schizophrenia

Almodóvar-Payá C, París-Gómez I, Latorre-Guardia M, Guardiola-Ripoll M, Catalán R, Arias B, Gallego C, Penadés R, Fatjó-Vilas M. Progress in Neuropsychopharmacololy & Biological Psychiatry. 2024 Oct 18;136:111175. doi: 10.1016/j.pnpbp.2024.111175. Epub ahead of print. PMID: 39426559.

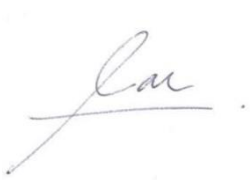
The Progress in Neuro-Psychopharmacology & Biological Psychiatry is an international and multidisciplinary journal which aims to disseminate research papers dealing with experimental and clinical aspects of neuro-psychopharmacology and biological psychiatry.

According to the Journal Citation Reports (Science Edition, 2023), the impact factor of the journal is 5.3 and it is ranked in the 90.5% percentile of the area of Pharmacology & Pharmacy, which corresponds to the first decile (D1).

The doctoral candidate has participated in the study conception, DNA extraction, and sample processing for genotyping and methylation analyses. Also, the

candidate has led the data curation, the statistical analysis, the interpretation and the visual presentation of the findings. Also, the doctoral student wrote the first version of the draft and conducted the revision and edition of the subsequent versions.

As the director and co-director of this thesis, we confirm the quality and originality of the articles published and that no co-author of the studies here presented has implicitly or explicitly used the results to elaborate another doctoral thesis.

Director	Co-director and tutor
	
Mar Fatjó-Vilas Mestre	Bárbara Arias Sampériz

4. Publications

Section I: The role of *NRN1* genetic variability in schizophrenia risk, clinical expression, intermediate phenotypes, and its interactions with related genes

Study I.I

NRN1 Gene as a Potential Marker of Early-Onset Schizophrenia: Evidence from Genetic and Neuroimaging Approaches

Almodóvar-Payá C, Guardiola-Ripoll M, Giralt-López M, Gallego C, Salgado-Pineda P, Miret S, Salvador R, Muñoz MJ, Lázaro L, Guerrero-Pedraza A, Parellada M, Carrión MI, Cuesta MJ, Maristany T, Sarró S, Fañanás L, Callado LF, Arias B, Pomarol-Clotet E, Fatjó-Vilas M

International Journal of Molecular Sciences. 2022 Jul 5;23(13):7456

doi: 10.3390/ijms23137456. PMID: 35806464

International Journal of
Molecular Sciences

MDPI

Article

NRN1 Gene as a Potential Marker of Early-Onset Schizophrenia: Evidence from Genetic and Neuroimaging Approaches

Carmen Almodóvar-Payá [1,2,†], Maria Guardiola-Ripoll [1,2,†], Maria Giralt-López [3,4], Carme Gallego [5], Pilar Salgado-Pineda [1,2], Salvador Miret [2,6,7], Raymond Salvador [1,2], María J. Muñoz [8], Luisa Lázaro [2,9,10,11], Amalia Guerrero-Pedraza [1,8], Mara Parellada [2,12,13,14], María I. Carrión [15], Manuel J. Cuesta [16,17], Teresa Maristany [18], Salvador Sarró [1,2], Lourdes Fañanás [2,19,20], Luis F. Callado [2,21,22], Bárbara Arias [2,19,20], Edith Pomarol-Clotet [1,2,*] and Mar Fatjó-Vilas [1,2,19,*]

1 FIDMAG Germanes Hospitalàries Research Foundation, 08830 Sant Boi de Llobregat, Barcelona, Spain; calmovodar@fidmag.com (C.A.-P.); mguardiola@fidmag.com (M.G.-R.); psalgado@fidmag.com (P.S.-P.); rsalvador@fidmag.com (R.S.); aguerrero.hbmenni@hospitalarias.es (A.G.-P.); ssarro@fidmag.com (S.S.)
2 Instituto de Salud Carlos III, Biomedical Research Network in Mental Health (CIBERSAM), 28029 Madrid, Madrid, Spain; smiret@gss.cat (S.M.); llazaro@clinic.cat (L.L.); parellada@hggm.es (M.P.); lfananas@ub.edu (L.F.); lf.callado@ehu.eus (L.F.C.); barbara.arias@ub.edu (B.A.)
3 Departament de Psiquiatria, Hospital Universitari Germans Trias i Pujol (HUGTP), 08916 Badalona, Barcelona, Spain; mariagiralt.germanstrias@gencat.cat
4 Departament de Psiquiatria i Medicina Legal, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Barcelona, Spain
5 Department of Cell Biology, Molecular Biology Institute of Barcelona (IBMB-CSIC), 08028 Barcelona, Barcelona, Spain; cggbmc@ibmb.csic.es
6 Centre de Salut Mental d'Adults de Lleida, Servei de Psiquiatria, Salut Mental i Addiccions, Hospital Universitari Santa Maria de Lleida, 25198 Lleida, Lleida, Spain
7 Institut de Recerca Biomèdica (IRB), 25198 Lleida, Lleida, Spain
8 Complex Assistencial en Salut Mental Benito Menni, 08830 Sant Boi de Llobregat, Barcelona, Spain; mmunoz.hbmenni@hospitalarias.es
9 Department of Child and Adolescent Psychiatry and Psychology, Institute of Neurosciences, Hospital Clinic de Barcelona, 08036 Barcelona, Barcelona, Spain
10 Departament de Medicina, Universitat de Barcelona (UB), 08036 Barcelona, Barcelona, Spain
11 Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036 Barcelona, Barcelona, Spain
12 Servicio de Psiquiatría del Niño y del Adolescente, Hospital General Universitario Gregorio Marañón, 28007 Madrid, Madrid, Spain
13 Instituto de Investigación Sanitaria del Hospital Gregorio Marañón (IiSGM), 28007 Madrid, Madrid, Spain
14 Departamento de Psiquiatría, Facultad de Medicina, Universidad Complutense, 28040 Madrid, Madrid, Spain
15 Hospital Sant Rafael, 08035 Barcelona, Barcelona, Spain; micarrion.hsrafael@hospitalarias.es
16 Servicio de Psiquiatría, Hospital Universitario de Navarra, 31008 Pamplona, Navarra, Spain; mj.cuesta.zorita@navarra.es
17 Instituto de Investigación Sanitaria de Navarra (IdiSNA), 31008 Pamplona, Navarra, Spain
18 Departament de Diagnòstic per la Imatge, Hospital Sant Joan de Déu Fundació de Recerca, 08950 Esplugues de Llobregat, Barcelona, Spain; tmaristany@sjdhospitalbarcelona.org
19 Departament de Biologia Evolutiva, Ecología i Ciències Ambientals, Universitat de Barcelona (UB), 08028 Barcelona, Barcelona, Spain
20 Institut de Biomedicina de la Universitat de Barcelona (IBUB), 08028 Barcelona, Barcelona, Spain
21 Department of Pharmacology, University of the Basque Country, UPV/EHU, 48940 Leioa, Bizkaia, Spain
22 Biocruces Bizkaia Health Research Institute, 48903 Barakaldo, Bizkaia, Spain
* Correspondence: epomarol-clotet@fidmag.com (E.P.-C.); mfatjo-vilas@fidmag.com (M.F.-V.)
† These authors contributed equally to this work.

Abstract: Included in the neurotrophins family, the Neuritin 1 gene (*NRN1*) has emerged as an attractive candidate gene for schizophrenia (SZ) since it has been associated with the risk for the disorder and general cognitive performance. In this work, we aimed to further investigate the association of *NRN1* with SZ by exploring its role on age at onset and its brain activity correlates. First, we developed two genetic association analyses using a family-based sample (80 early-onset (EO) trios (offspring onset $\leq$ 18 years) and 71 adult-onset (AO) trios) and an independent case–control sample (120 healthy subjects (HS), 87 EO and 138 AO patients). Second, we explored the effect of *NRN1* on brain activity during a working memory task (N-back task; 39 HS, 39 EO and 39 AO;

check for updates

Citation: Almodóvar-Payá, C.; Guardiola-Ripoll, M.; Giralt-López, M.; Gallego, C.; Salgado-Pineda, P.; Miret, S.; Salvador, R.; Muñoz, M.J.; Lázaro, L.; Guerrero-Pedraza, A.; et al. *NRN1* Gene as a Potential Marker of Early-Onset Schizophrenia: Evidence from Genetic and Neuroimaging Approaches. *Int. J. Mol. Sci.* **2022**, *23*, 7456. https://doi.org/10.3390/ijms23137456

Academic Editor: Marco Fiore

Received: 29 April 2022
Accepted: 28 June 2022
Published: 5 July 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

matched by age, sex and estimated IQ). Different haplotypes encompassing the same three Single Nucleotide Polymorphisms(SNPs, rs3763180–rs10484320–rs4960155) were associated with EO in the two samples (GCT, TCC and GTT). Besides, the GTT haplotype was associated with worse N-back task performance in EO and was linked to an inefficient dorsolateral prefrontal cortex activity in subjects with EO compared to HS. Our results show convergent evidence on the *NRN1* association with EO both from genetic and neuroimaging approaches, highlighting the role of neurotrophins in the pathophysiology of SZ.

Keywords: schizophrenia-spectrum disorders; *NRN1*; age at onset; working memory; functional magnetic resonance imaging (fMRI)

1. Introduction

Substantial evidence highlights the importance of the genetic component in the aetiology of schizophrenia (SZ), with an estimated heritability of around 65–79% [1,2]. Indeed, genome-wide association studies (GWAS) have confirmed SZ's polygenic architecture, resulting from the aggregated effect of low impact variants and reporting an SNP-based heritability of 24% [3]. Moreover, genomic data converge into identifiable biological pathways involved in neurodevelopment, particularly highlighting the mechanism of synaptic plasticity [3–5].

However, in the search for specific genetic factors related to SZ, studies face several challenges that arise from the genetic and phenotypic complexity of the disorder [6]. Then, it has been suggested that combining complementary designs, such as family-based and case–control, would clear the way to dissect the genetic influences of the disorder [7]. In this sense, family-based genetic association designs have the advantage of reducing the problem of stratification and spurious association when compared to case–control studies, while the latter usually allow for larger sample sizes [8].

In addition to the design, the approaches to the phenotypic complexity of SZ have also been considered using narrower phenotypes with particular aetiological significance to reduce the heterogeneity and identify specific genetic factors associated with the disorder. One of these phenotypes is the age at onset, which shows a heritability of around 33% [9]. Notably, early age at onset (EO) has captured much attention because it is considered a marker of a higher genetic liability than adult-onset (AO) [10,11]. The EO term includes cases with onset up to 18 years of age and, despite being arbitrary, roughly corresponds with the upper age cut-off in most published studies of child and adolescent psychosis [12]. In this support, EO subjects show a higher familial aggregation of SZ and other mental disorders [13], poorer premorbid adjustment [14] and neurocognitive performance [15], more severe outcomes [16,17] and more prominent alterations in neurodevelopmental trajectories than AO forms [18]. The few GWAS focused on searching for genetic loci associated with age at onset in SZ have confirmed that some variants overlap with those conferring risk for SZ, while others are pure modifiers [19–22]. Remarkably, EO patients present higher SZ polygenic risk scores than their siblings, with the scores effectively predicting an earlier age at onset [23]. Interestingly, the variants associated with an earlier age at onset converge into molecular networks related to nervous system development, the regulation of axon extension, modulation of glial proliferation, molecular transport, and cell-to-cell signalling and interactions [20,22].

Among genes with pivotal roles through all stages of the brain's formation, there is the Neuritin 1 gene (*NRN1*, 6p25.1) (see review [24]), which is highly expressed in the hippocampus, the cerebral cortex and the cerebellum [25,26] in an activity-dependent manner [27,28]. Although the Nrn1 receptor and its downstream signalling effectors are still being studied, it seems that Nrn1 regulates synaptic excitability through the activation of the insulin receptor (IR) and its downstream signalling pathways [29,30]. Consequently, inadequate Nrn1 sustenance could translate into the abnormal formation of synapses, a

reduced capacity to perform adaptive responses and, in turn, a higher risk of developing a mental disorder. In fact, the interest in the role of Nrn1 in SZ has been motivated by several studies, which have evidenced its impact on cognitive function through synaptic plasticity mechanisms. From cell- and animal-based approaches, it has been shown that the viral-mediated overexpression of *NRN1* in different models (unpredictable stress-induced rat depression model, mice exposed to low-frequency electromagnetic fields and an Alzheimer's disease model Tg2576 mouse) prevents the atrophy of dendrites and spines and improves associated behaviours, such as anxiety, depression, deficits in novel object recognition, learning and memory [31–33]. Additionally, the expression of *NRN1* has been shown to increase in the hippocampus of mice exposed to electroconvulsive therapy and fluoxetine administration [32,34]. These studies highlight the potential therapeutic use of *NRN1* in disorders associated with loss of cognitive function, such as SZ, and appeal for a better understanding of its molecular mechanisms. From human-based studies, *NRN1* has been already defined as a candidate gene for SZ since specific allelic variants have been associated with an incremented risk of developing the disorder. Moreover, *NRN1* has also been described as a modifier of the SZ phenotype due to its association with patients' general cognitive ability and age at onset [35,36]. This suggests that *NRN1* may be involved in critical mechanisms of brain development, particularly in those most susceptible to the earlier onset of the symptoms.

Neuroimaging data can provide evidence on how the genetic actors underlying an earlier age at onset contribute to the neurobiology of the disorder [37]. In this sense, functional neuroimaging studies focused on exploring the brain activity during working memory (WM) tasks (related to the capacity to retain and use mental items during a short period) are of particular interest. Subtle WM deviances have been described in the healthy siblings of subjects with SZ compared to healthy subjects (HS) in studies focused on cognition [38], brain activity [39] and connectivity [40]. This suggests that WM alterations in SZ are genetically influenced. Indeed, disabilities in this cognitive domain are considered to be a core feature of SZ [41] and have been reported to be even more severe in EO patients [15].

Several studies based on functional magnetic resonance neuroimaging (fMRI) and exploring brain networks supporting WM have consistently described frontoparietal differences in individuals with SZ when compared to HS. Most of these studies described the decreased activity of the dorsolateral prefrontal cortex (DLPFC), the ventrolateral prefrontal cortex (VLPFC) and anterior cingulate cortex (ACC) as a key mechanism of WM dysfunction [42]. The few functional neuroimaging studies specifically focused on individuals with EO have reported similar patterns of abnormal activations in these regions of the prefrontal cortex (e.g., VLPFC, DLPFC, and ACC) plus some limbic and temporal regions [43–48]. However, those studies are scarce, in part, due to the low rate of EO, which represents only about 8% among individuals with SZ [49], and they have reported inconsistent findings regarding the direction of the results. In this context, the study of the genetic WM correlates in individuals with EO forms is particularly pertinent since it could offer insights into the impact of genetic architecture on brain activity and, ultimately, on the clinical manifestation of SZ.

Considering all the above-cited evidence, we hypothesised that the polymorphic variability of *NRN1* would be differentially associated with the risk of developing EO forms of SZ compared to AO. We developed this study by combining different designs (family-based and case–control sample approaches) to provide robustness to our findings. Additionally, we hypothesised that those genetic variants conferring risk for EO would differentially impact WM-related brain activity.

2. Results

2.1. Genetic Association Analyses

2.1.1. Family-Based

The genotypes/alleles counts and frequencies of EO/AO offspring and parents are listed in Supplementary Table S1. As shown in Table 1, within EO families, the GCT haplotype including SNP6, SNP7 and SNP8 (HAP678) was significantly under-transmitted from parents to affected offspring (p_{perm} = 0.03). Our analyses did not reveal any association between the genetic variability at *NRN1* with the risk for AO SSD, neither in the allelic, genotypic or haplotype approach.

Table 1. Significant genetic association results within early-onset (EO) family-based and EO case–control samples. At the top row, for the family-based approach, there are the transmitted and not-transmitted haplotype (HAP) counts from heterozygous parents to affected offspring for family-based analyses. Below, for the case–control approach, the frequency (%) in healthy subjects (HS) and schizophrenia spectrum disorders (SSD) are given with the risk genotype placed last. The odds ratio (OR) associated with the genotype and the confidence interval (CI 95%) are also reported. The empirical *p*-values obtained after 10,000 permutation procedures (p_{perm}) for the Transmission Disequilibrium Test (TDT) or the logistic regression (additive model) are shown.

SNPs	Haplotype	Transmitted EO SSD	Not Transmitted EO SSD	OR (CI 95%)	TDT; p_{perm}
HAP678	GCT	13	27	0.48 (0.25–0.93)	4.90; 0.03
SNPs	**Genotypes Haplotypes**	**Frequency EO SSD**	**Frequency HS**	**OR (CI 95%)**	**Wald; p_{perm}**
SNP6	TT/TG/GG	11 (0.13)/40 (0.48)/33 (0.39)	31 (0.26)/58 (0.49)/30 (0.25)	1.68 (1.01–2.57)	2.39; 0.02 [a,b]
SNP7	CC/CT/TT	43 (0.50)/35 (0.41)/8 (0.09)	78 (0.66)/35 (0.29)/6 (0.05)	1.69 (1.06–2.71)	2.19; 0.03 [b]
SNP8	CC/CT/TT	14 (0.17)/41 (0.49)/29 (0.35)	33 (0.29)/58 (0.51)/23 (0.20)	1.66 (1.08–2.56)	2.31; 0.02 [a,c]
HAP678	TCC	0.37	0.50	0.59 (0.39–0.89)	6.44; 0.01
HAP678	GTT	0.30	0.20	1.70 (1.08–2.67)	5.28; 0.02

[a] The genotypic model was also significant ($p_{perm} < 0.05$). [b] The dominant model was also significant ($p_{perm} < 0.05$). [c] The recessive model was also significant ($p_{perm} < 0.05$).

2.1.2. Case–Control

The distribution of the genotypes/alleles in HS, EO and AO subjects is reported in Supplementary Table S2. As exposed in Table 1, we observed a significant association of SNP6 G allele (p_{perm} = 0.02), SNP7 T allele (p_{perm} = 0.03) and SNP8 T allele (p_{perm} = 0.02) with EO SSD under an additive model. We also identified an association of two haplotypes including SNP6, SNP7 and SNP8 (HAP678) with the risk for EO SSD, which was in line with the SNP-based results. The GTT haplotype was significantly more frequent in subjects with EO SSD than in HS (p_{perm} = 0.02), while the TCC was more frequent in HS (p_{perm} = 0.01). Other 2-SNP and 4-SNP haplotypes containing these same variants were also associated with the risk for EO (Supplementary Table S3). Our analyses did not reveal any effect of genetic variability at *NRN1* on the risk for AO SSD, in any of the tested models (allelic, genotypic and haplotypic).

2.2. Neuroimaging Genetic Association Analyses

2.2.1. N-Back Functional Response

The three groups (HS, EO and AO) showed typical WM-related activation and deactivation patterns (Supplementary Figures S1–S3). In addition, both EO and AO exhibited a deactivation failure when compared to HS in overlapping regions involving bilateral structures, such as the frontal gyrus (superior, medial and inferior orbital part), the olfactory area, the rectus and the anterior cingulate and the paracingulate gyri, as well as right structures, such as the superior and middle temporal gyrus, the parahippocampal gyrus, the hippocampus, the amygdala, the fusiform gyrus and the caudate nucleus (Supplementary Figure S4).

We detected a significant diagnosis x HAP678 (GTT) interaction for the EO vs. HS comparison in the 2-back vs. 1-back contrast in one cluster located at the superior and middle frontal gyrus, regions of the DLPFC (316 voxels, peak activation at MNI coordinates [−34,42,42], Zmax = 4.54, $p = 0.0025$, Figure 1A). To further interpret this result, mean activity scores for the 1-back and 2-back contrasts were plotted. As shown in Figure 1B, HS exhibited a cluster mean activity of around zero for the two contrasts, irrespective of their haplotypic profile. Subjects with EO without the risk haplotype showed a pattern towards increased cluster activity from 1-back to 2-back contrasts, whereas those patients carrying the risk haplotype presented a pattern towards decreased cluster activity. We did not observe any significant interaction on brain activity when we compared HS and AO groups.

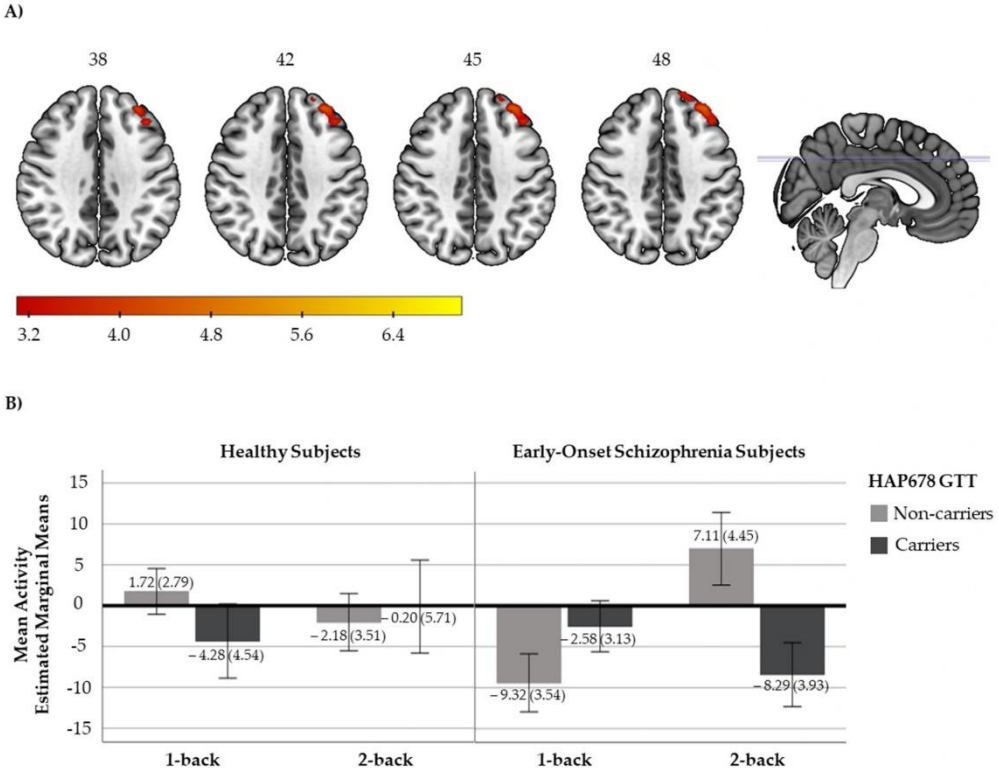


Figure 1. (**A**) Axial view of the brain showing the significant cluster derived from the diagnosis x *NRN1* HAP678 GTT analysis in the 2-back vs. 1-back contrast. A sagittal view with the marks of the cross slices is also included. The right side of the image represents the right side of the brain. The MNI coordinates are given for the shown slices. Units of the bar correspond to the β values of the regression, standardised to Z scores. (**B**) Bar plots with the cluster mean activity (estimated marginal means and ±2 standard errors (se)) for healthy subjects (HS; left, non-carriers: n = 27, carriers: n = 10) and subjects with early-onset schizophrenia (EO; right, non-carriers: n = 19, carriers: n = 20).

2.2.2. N-Back Behavioural Response

First, subjects with EO exhibited a globally poorer performance of the N-back task than HS in both difficulty levels (mean (SD) d'1: EO 3.07 (1.16) and HS 4.14 (0.68), F = 13.00, $p = 0.001$; mean (SD) d'2: EO 2.06 (0.90) and HS 3.41 (0.88), F = 27.52, $p < 0.001$). While both

groups showed different scores at the two levels of the task, their degree of decrease in performance from the 1-back to 2-back was similar (F = 1.55, $p = 0.22$).

Second, AO and HS exhibited a similar performance in the low memory load condition, but their performance diverged in the high memory load condition (mean (SD) d'1: AO 3.81 (0.90) HS 4.14 (0.68), F = 0.33, $p = 0.57$; mean (SD) d'2: AO 2.48 (0.82) and HS 3.41 (0.88), F = 15.27, $p < 0.001$). Then, as the performance of the two groups was similar for the 1-back, the degree of change from the 1-back to -back was more pronounced in subjects with AO than HS (F = 8.11, $p = 0.01$).

Third, EO performance at the low memory load condition was modulated by *NRN1* haplotypic variability. Subjects carrying the HAP678 GTT showed a poorer performance when compared to those without the risk haplotype (mean (SD) d'1: non-carriers 3.67 (0.22) and carriers 2.86 (0.19), F = 5.66, $p = 0.02$) (Figure 2). No effect of *NRN1* haplotypic variability on task performance was detected in either HS or AO subjects.

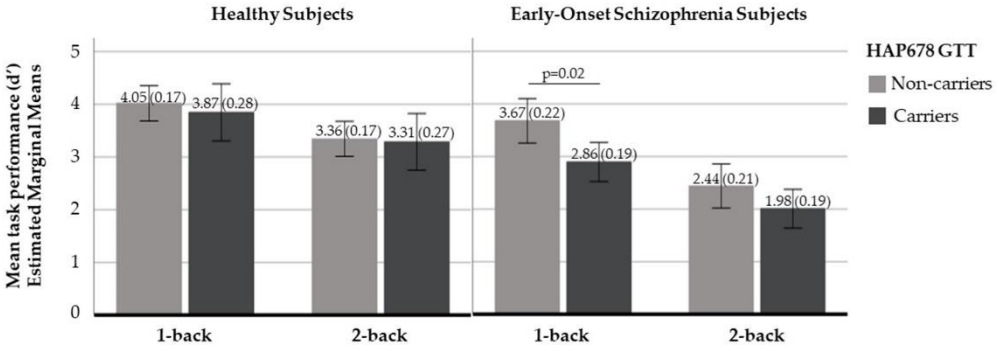


Figure 2. Bar plots with mean performance (estimated marginal means and ±2 standard errors(se)) for healthy subjects (HS; left, non-carriers: n = 27, carriers: n = 10) and subjects with early-onset schizophrenia (EO; right, non-carriers: n = 19, carriers: n = 20) by *NRN1* HAP678 GTT.

3. Discussion

In this study, we combined genetic association and neuroimaging approaches to deepen into the role of *NRN1* in the age at onset of SZ. Regarding the genetic association approach, our study adds to the only two previous studies on the association of the *NRN1* gene with SZ and other disorders within the spectrum [35,36], but it is the first to be developed through family-based and case–control designs in two independent samples. Our results, derived from the two samples, suggest that the variability at *NRN1* may explain a modest proportion of the risk of EO. Concerning the neuroimaging approach, our study represents the first to explore WM neural correlates of *NRN1*. Our findings indicate that *NRN1* variants conferring risk for SZ also have an effect on the performance of the N-back task, specifically within EO subjects. Additionally, we report brain activity differences between EO subjects and HS located at the DLPFC conditional to the same genetic variants.

Our genetic association analyses identified different SNPs and haplotypes at *NRN1* associated with EO in SSD. We detected a significant under-transmission of the HAP678 GCT from parents to affected offspring, specifically in EO families. In parallel, through a case–control approach, we identified the effect of SNP6, 7 and 8 on the risk for EO SSD and the association of a risk haplotype encompassing these same polymorphisms HAP678 GTT. On the contrary, we did not detect any association with AO SSD. Therefore, our data converge into the view of polymorphisms at *NRN1* (SNP6, SNP7, SNP8) as a relevant genetic variability source in modifying the neurodevelopment processes related to the earlier emergence of these disorders. It is of note that these SNPs have been previously associated with the risk for SZ in the two-preceding works [35,36]. Moreover, one of these studies also identified a role of *NRN1* in age at onset of SSD [36]. However, our results

should be interpreted in the context of the polygenic architecture of these disorders, as the effect of the SNPs and haplotypes is small (see the corresponding ORs). Still, this evidence suggests that those genes that influence brain development, such as *NRN1*, may modify illness traits, such as age at onset, and ultimately affect the risk for these disorders.

Due to the few studies focused on examining the association of the *NRN1* gene with SZ, data from whole-genome approaches must be taken into consideration for the further interpretation of our results. First, different genetic linkage studies mapping SZ to a genomic location pointed towards the association of chromosome region 6p24-25 and highlighted *NRN1* as a positional candidate gene [50–53]. However, as far as we know, *NRN1* has not appeared as a significant locus in the latest genome-wide association studies [3]. These negative results could be explained due to the modifier properties of *NRN1*, which means that, as our results suggest, *NRN1* modulates SSD phenotype through its impact on age at onset. Some linkage and whole-genome studies that specifically aimed to identify modifier loci related to the age at onset in SZ have highlighted the chromosome region 6p24 and some *NRN1* neighbouring intergenic variants with putative regulatory roles on its expression [21,54]. Additionally, whole-genome approaches have also linked *NRN1* and SZ through epigenetic mechanisms. In this respect, Pidsley et al., 2014 [55] identified, through a methylomic approach in human post-mortem prefrontal cortex samples, a wide genetic region that is hypomethylated in patients compared to controls, spanning the body of *NRN1*. This result suggests that *NRN1* could be differentially expressed in the prefrontal cortex of subjects with SZ, a brain region repetitively described to be altered in this disorder [56].

To explore the neurobiological translation of the observed genetic variants conferring a higher risk for EO SZ, we developed a neuroimaging genetic study in a matched case–control sub-set. Our functional data suggest that the risk haplotype (HAP678 GTT) that is associated with the earlier emergence of the disorder is also associated with DLPFC activity changes within this group of patients. Concretely, through the analysis of differences between the two levels of the N-back task (2-back vs. 1-back contrast), we observed that EO subjects not carrying the risk haplotype changed DLPFC activity towards activation in response to the task's increasing difficulty. At the same time, those carrying the risk haplotype were prone to decreased activity. The previous few studies exploring whole-brain activity differences between subjects with EO and HS have reported inconsistent findings regarding the implicated regions. Some reported reduced activation of the left VLPFC and extrastriate visual cortex [43], while others described VLPFC hyperactivation [47]. Other works reported the reduced engagement of the DLPFC, the ACC, frontal operculum and inferior and posterior parietal and caudate [44–46], contrary to other investigations that suggested increased activations in the ACC, medial temporal lobe structures, the insula and bilateral lateral temporal lobes [48]. In this respect, our results shed light on those controversial findings, as they provide evidence that genetic factors, in this case, the *NRN1* gene, could be underlying these differences in DLPFC activity.

To interpret our functional results in the DLPFC, it is important to integrate brain activity findings with N-back behavioural data. On the one hand, the sustained activation of the prefrontal circuits is considered a key mechanism for executing high-memory-load tasks [57]. On the other hand, it is also known that the degree of change in DLPFC activity is related to the cognitive effort needed to perform the task. In other words, if the computational cost is unlikely to result in the accurate performance of the task, the prefrontal resources get disengaged [58]. In this sense, EO subjects not carrying the risk haplotype displayed a better performance at low memory load and exhibited a higher degree of DLPFC modulation towards activation in response to a higher memory load level. This result suggests that EO subjects without the risk haplotype may use greater prefrontal resources in response to task difficulty increase than those with the risk haplotype, who seem to reach activation and performance peaks at a lower processing load.

On the whole, our functional and behavioural results align with the preceding evidence linking *NRN1* and cognitive performance in SZ [35,36] and executive function in HS [59].

Furthermore, they suggest that these prefrontal networks of sustained activation during WM, in which *NRN1* appears to have a relevant role, might be especially sensitive to the earlier onset of psychosis. The specific effects in EO forms of SZ seem reasonable since several investigations have described adolescence as a crucial period for the development of the prefrontal cortex [60] and the reorganisation of the WM network [61,62]. This is also supported by several studies showing the greater recruitment of WM regions in adults than in children [63]. In this view, the earlier onset of the disorder might strongly impact the neural trajectories associated with WM development, potentially leading to WM-characteristic impairments in EO compared to AO [64]. Interestingly, regarding the specific role of DLPFC activity and *NRN1* as potential markers of EO, a recent study using an innovative transcriptomic approach defined two molecularly distinct subgroups of subjects with SZ [65]. The first presented a DLPFC transcriptome very similar to that of HS, while the second exhibited a strikingly different DLPFC transcriptome, with the *NRN1* gene included among the differentially expressed genes. These data suggest that fundamental biologic differences exist between subjects diagnosed with SZ. Thus, our results on the modulation effect of *NRN1* haplotypic variability on brain function and performance contribute to bridging the gap between the role of *NRN1* in synaptic plasticity processes and the pathophysiological mechanisms underlying SZ.

Towards a further understanding of such mechanisms, considering the putative effects of the analysed polymorphic sites on gene expression regulatory mechanisms represents a valuable resource to provide additional meaning and importance to our association data. Among the three variants encompassed by the HAP678 risk haplotype (LD = 0.99 in both samples), data from the RegulomeDB and Haploreg highlight the functional effects of SNP6 (rs3763180). There is evidence that this variant could modify the histone enhancer and promoter marks in the brain, contributing to the chromatin state at this locus. Moreover, this variant is predicted to alter motifs that overlap the recognition sequence of different transcription factors, such as the alpha isoform of the CCAAT-enhancer binding proteins (C/EBP), PBX homeobox 3 (PBX3) and the Neuron-Restrictive Silencing Factor (NRSF). Interestingly, the change of a T to a G in that position is linked to increased NRSF affinity, implicated in the programming of stress-sensitive neurons by neonatal experience through epigenetic mechanisms, promoting resilience to stress-related emotional disorders [66]. The other two variants included in the HAP678 presented a lower functionality score; still, data show their putative modulatory effects on the affinity of some transcription factors. For instance, the SNP7 (rs10484320) is suggested to modify the binding of the TATA-binding protein (TBP) and PU.1. The TBP has been associated with the risk for SZ, age at onset and prefrontal function [67]. Additionally, higher levels of the transcription factor PU.1, required for the development of the immune system, have been detected in post-mortem brain samples from individuals diagnosed with SZ compared to HS [68]. Additionally, accordingly to Brainiac data, when the effect of these three SNPs stratifies the expression of *NRN1* transcripts, genotype-based differences emerge in the hippocampus, and a trend effect is detected in the cortex. These lines of evidence suggest putative molecular mechanisms by which the SNPs included in the HAP678 may affect the complex phenotype of SZ. Nevertheless, further functional data on these SNPs are needed to fully characterise their impact on the underlying mechanisms that connect *NRN1* and age at onset of psychosis.

Finally, our study should be interpreted in the context of some limitations. First, regarding our genetic association approach, the samples could be considered to be relatively small. However, according to the statistical power of our analyses and after multiple testing correction procedures, we concurrently identified, in two independent samples, the impact of *NRN1* genetic variants on the risk for the earlier onset of SZ. Second, all the variants included in the present study are polymorphic; however, it is known that a certain proportion of the variance in genetic liability of SZ is also accounted for by rare variants [69,70]. Therefore, different approaches analysing the combined role of common and low-frequency variants along *NRN1* gene on SZ would be of potential interest. Third,

while the present study has not directly analysed the functional consequences of the *NRN1* variants associated with EO, our results and the available functional data suggest the need for cell-based studies integrating genetic variability information. Fourth, in the case of neuroimaging approaches, although we compared EO subjects and HS, patients were scanned in their adulthood, years after the onset of the illness. Therefore, illness duration and related clinical variables could have affected the results. Based on this, we checked the possible impact of illness duration or medication on the mean activity and the d' scores through regressions. While we cannot completely rule out the effect of these variables the lack of significance suggests that our results are not modulated by them. Additionally, it should be underlined that activation differences at prefrontal regions have been observed in unaffected first relatives of SZ patients [39], individuals at clinical high risk for psychosis [71] and individuals with treatment-naïve first episode psychosis [72], suggesting that this pattern may represent an intrinsic feature of SZ rather than a medication effect. Lastly, the absence of representation of diverse ethnic groups and the low proportion of females within our EO group hampers the extrapolation of our results and demands the need for new studies in larger samples with equal representation of those populations.

4. Materials and Methods

4.1. Sample

This study included 798 individuals (Table 2). Two independent samples were used to develop separate genetic association analyses: (i) Sample 1 comprised 151 trios (with an offspring diagnosed with schizophrenia spectrum disorders (SSD) plus 302 healthy parents), (ii) Sample 2 consisted of 225 independent patients diagnosed with SSD and 120 HS. Also, from Sample 2, a sub-set of cases with SZ (39 EO and 39 AO) and HS (39) (matched by sex, age and estimated IQ) was selected to develop a neuroimaging genetic analysis (Sample 3). Participants were drawn from admissions to both Child and Adolescent and Adult Psychiatric Units. All HS were recruited from non-medical staff working in the hospital, their relatives and acquaintances, plus independent sources in the community.

All patients were evaluated by experienced psychiatrists and met the DSM-IV-TR criteria for SSD, including schizophrenia, schizophreniform disorder, schizoaffective disorder and psychosis disorder not otherwise specified (Table 2). Patients up to 17 years old were diagnosed following Kiddie Schedule for Affective Disorders and Schizophrenia (KS-DAS, [73]), while the Comprehensive Assessment of Symptoms and History (CASH, [74]) or the Structured Clinical Interview for DSM Disorders (SCID, [75]) was used for adult patients. Age at onset of the first episode was determined using these clinical schedules and/or the Symptom Onset in Schizophrenia inventory (SOS, [76]). Following previous studies [12], subjects with SSD were classified as either EO when the first episode occurred before or at 18 years, or as adult-onset AO when presented at age 19 or older.

The general exclusion criteria included an age above 65 years, major medical illnesses that could affect brain functions, substance-induced psychotic disorder, neurological conditions and having had at least one parent not from European ancestry. Moreover, all the relatives and HS underwent a clinical interview on personal and/or familial psychiatric history using Family Interview for Genetic Studies (FIGS) [77] and those who reported a personal history of mental illness or treatment with psychotropic medication were excluded.

For the subjects included in the neuroimaging study, the exclusion criteria also included an estimated IQ under 70, left manual dominance, and a history of head trauma with loss of consciousness. The evaluation of patients comprised the Positive and Negative Symptoms Scale (PANSS), while the estimated IQ of both patients and controls, was assessed using the Word Accentuation Test [78], which requires the pronunciation of 30 low-frequency Spanish words whose accents were removed.

All participants provided written consent after being informed about the study procedures and implications. In the case of patients below the age of 18, written consent was also obtained from their parents. The study was performed following the guidelines of the

institutions involved and was approved by the local ethics committee of the centre. All procedures were carried out according to the Declaration of Helsinki.

Table 2. Sociodemographic and clinical information for the family-based and case-control samples included in the genetic association and neuroimaging analyses. Data for patients are given separately for subjects with early-onset (EO) and adult-onset (AO) schizophrenia spectrum disorders (SSD). Number (percentage in brackets) are shown for qualitative variables. Mean scores (standard deviation in brackets) are provided for quantitative variables. Illness duration refers to years. The psychopathology was assessed using the Positive and Negative Symptoms Scale (PANSS). Treatment was defined by chlorpromazine equivalence (CPZE). Those not significant values are not reported (n.s.).

Sample 1: Family-Based [a] **(n = 453)**	**AO Offspring (n = 71)**	**EO Offspring (n = 80)**		**AO Parents (n = 142)**	**EO Parents (n = 160)**	
Male	58 (81.70)	54 (67.50)	n.s.	71 (50.00)	80 (50.00)	n.s.
Age at interview	27.45 (5.03)	18.04 (4.94)	$t = -11.51$, $p < 0.001$	50.04 (7.97)	58.34 (8.44)	$t = -7.27$, $p < 0.001$
Age at onset	23.24 (4.24)	15.41 (2.12) [d]	$t = -13.35$, $p < 0.001$	–	–	–
Sample 2: Case-control [b] **(n = 345)**	**AO Subjects (n = 138)**	**EO Subjects (n = 87)**		**Healthy Subjects (n = 120)**		
Male	93 (67.40)	67 (77.00)	n.s.	60 (50.00)		$\chi^2 = 17.13$, $p < 0.001$
Age at interview	41.97 (10.03)	39.79 (10.87)	n.s.	38.24 (11.21)		$F = 3.45$; $p = 0.033$ [e]
Age at onset	25.12 (5.86)	16.38 (2.00)	$t = 16.11$, $p < 0.001$	–		–
Sample 3: Neuroimaging [c] **(n = 117)**	**AO Subjects (n = 39)**	**EO Subjects (n = 39)**		**Healthy Subjects (n = 39)**		
Male	37 (94.87)	37 (94.87)	n.s.	37 (94.87)		n.s.
Age at interview	39.49 (1.90)	39.30 (1.87)	n.s.	38.43 (1.78)		n.s.
Age at onset	24.56 (0.80)	16.85 (0.26)	$t = 9.17$; $p < 0.001$	-		-
Illness duration	14.92 (11.01)	22.46 (11.31)	$t = -2.98$; $p = 0.004$	-		-
PANSS total	68.72 (20.46)	80.05 (21.11)	$t = -2.60$; $p = 0.011$	-		-
CPZE	367.01 (188.83)	633.66 (304.39)	$t = -4.28$; $p < 0.001$	-		-

[a] The different SSD diagnoses were equally distributed between the AO and EO offspring ($\chi^2 = 1.271$, $p = 0.736$): Schizophrenia (AO n = 41 (57.75%); EO n = 47 (58.75%)), Schizophreniform (AO n = 13 (18.31%); EO n = 10 (12.50%)), Schizoaffective (AO n = 6 (8.45%); EO 7 (8.75%)) and Psychosis not otherwise specified (AO n = 11 (15.4%); EO n = 16 (20.00%)). [b] The different diagnoses were equally distributed between the AO and EO cases ($\chi^2 = 1.558$, $p = 0.212$): Schizophrenia (AO n = 130 (84.42%); EO n = 84 (90.32%)) and Schizoaffective (AO n = 24 (15.58%); EO n = 9 (9.80%)). [c] Sub-set of individuals coming from Sample 3. Patients included in the neuroimaging sample were all diagnosed with schizophrenia. [d] Information was available for 60% of patients but all were drawn from child and adolescent units which allowed their classification as EO. [e] The post-hoc analyses showed significant differences only between healthy subjects and subjects with AO SSD ($p = 0.010$).

4.2. Genotyping

Genomic DNA was obtained for all individuals either from buccal mucosa through cotton swabs or from peripheral blood cells by punction and extracted using an ATP Genomic DNA Mini Kit Tissue (Teknokroma Analítica, S.A., Sant Cugat del Vallès, Barcelona, Spain) or using a Realpure SSS Kit for DNA Extraction (Durviz, S.L.U, Valencia, Spain), respectively.

All Single Nucleotide Polymorphisms (SNPs) were determined via a fluorescence-based allelic discrimination procedure (Applied Biosystems Taqman 5′-exonuclease assays) using standard conditions.

The information about the SNPs is given in Table 3. The SNPs were selected based on two previous studies [35,36]. All SNPs had a minor allele frequency above 5% and were non-coding. As previous evidence suggests that non-coding variants exert important regulatory effects [79] and that such effects are particularly important in SZ [80], the functional consequences of the analysed SNPs were evaluated using different resources. First, HaploReg was used to obtain information about the impact of non-coding variants on chromatin state, protein binding, sequence conservation across mammals, regulatory motifs and expression (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php, [81], accessed on 1 June 2022). It showed that several SNPs (from rs12333117 to rs3763180) are classified as genetic promoters or enhancers in the brain tissue, based on histone marker data from the Epigenetic Roadmap. Moreover, all the variants, except for one (rs582186), are predicted to change the affinity of multiple regulatory motifs based on data from the

ENCODE project. Second, the Regulome DataBase (http://www.regulomedb.org/, [82], accessed on 1 June 2022), a model integrating functional genomic features, was used to obtain a functional probability score for each SNP. This score ranges from 0 to 1, with 1 being most likely to be a regulatory variant [83]. As shown in Table 3, several of the selected SNPs had a score above 0.61 (from rs12333117 to rs3763180). Third, the Brain eQTL Almanac (Braineac), which is a web-based (http://www.braineac.org, accessed on 1 June 2022) resource to access the UK Brain Expression Consortium (UKBEC) dataset, showed that the assessed *NRN1* transcripts are mainly expressed in the cortex, hippocampus and cerebellum. In addition, this tool was also used to evaluate the effect of the SNPs associated with EO forms of SZ on brain expression patterns (see Discussion).

Table 3. Information on the Single Nucleotide Polymorphisms (SNPs) at Neuritin 1 gene included in this study (*NRN1*, chromosome 6p25.1, from 5,997,999 to 6,007,605 bp, UCSC Genome Browser on Human Assembly GRCh38/hg38, http://genome.ucsc.edu/cgi-bin/hgTracks, accessed on 1 April 2022). The table includes dbSNP number, the chromosome and gene position, the alleles of each SNP, the minor allele frequency (MAF; described for all and EUR populations in the 1000 Genomes Project and the MAF observed in each sample included in the present study) and the functional score according to the Regulome Database.

SNPs		Chromosome Position	Gene Position	Alleles (Minor/Major)	MAF (All/Eur)	Family-Based MAF	Case-Control MAF	RegulomeDB Score [a]
SNP1	rs2208870	5,992,257	intergenic	G/A	0.33/0.34	0.33	0.33	0.61
SNP2	rs12333117	5,994,759	intergenic	T/C	0.35/0.40	0.43	0.38	0.61
SNP3	rs582186	6,001,148	downstream	A/G	0.45/0.62	0.61	0.40	0.61
SNP4	rs645649	6,004,726	intronic	C/G	0.45/0.64	0.64	0.38	0.61
SNP5	rs582262	6,007,758	intronic	C/G	0.30/0.48	0.28	0.27	0.70
SNP6	rs3763180	6,009,615	upstream	T/G	0.40/0.46	0.45	0.43	0.63
SNP7	rs10484320	6,010,204	upstream	T/C	0.15/0.22	0.26	0.24	0.16
SNP8	rs4960155	6,010,306	upstream	T/C	0.43/0.49	0.50	0.49	0.13
SNP9	rs9379002	6,012,158	intergenic	G/T	0.29/0.42	0.24	0.26	0.13
SNP10	rs9405890	6,012,488	intergenic	C/T	0.31/0.38	0.28	0.33	0.18
SNP11	rs1475157	6,016,936	intergenic	G/A	0.16/0.17	0.16	0.16	0.18

[a] This score ranges from 0 to 1, with 1 being most likely to be a regulatory variant.

All markers were in Hardy–Weinberg equilibrium in the two samples: the family-based (parents and offspring) and the case–control (subjects with SSD and HS). The total genotypic call rate was 95.48 and 98.85%, respectively.

4.3. fMRI Task Description and Acquisition Parameters

4.3.1. N-Back Task

Functional images were acquired while participants performed a sequential-letter version of the N-back task, which engages storage and executive processes related to attention and WM. Briefly, in this task, letters were presented sequentially in a random way, and the participants were required to press a button when the letter shown on the screen matched the one presented one step prior in the sequence (condition 1-back) or the one from two steps before in the sequence (condition 2-back). The two levels of memory load were presented in a block design manner. Each block consisted of 24 letters shown every 2 s (1 s on, 1 s off), and all blocks contained 5 letter repetitions located randomly within the blocks. Four 1-back and four 2-back blocks were presented in an interleaved way, and between them, a baseline stimulus (an asterisk flashing with the same frequency as the letters) was presented for 16 s. Letters were displayed in green for 1-back blocks and in red for 2-back blocks to identify which condition had to be performed. All participants went through a training session before entering the scanner.

4.3.2. N-Back Performance Data

To measure the behavioural performance of the task, we used the signal detection theory index sensitivity, d' scores [84]. Higher values of d' indicate a better ability to

discriminate between targets and distractors, while negative values indicate that subjects are not performing the task. Then, all the individuals included in the analyses had positive d' values (d'1 for 1-back and d'2 for 2-back).

4.3.3. fMRI Acquisition Parameters

The fMRI data acquisition was performed with a GE Sigma 1.5-T scanner (General Electric Medical Systems, Milwaukee, WI, USA) at Hospital Sant Joan de Déu (Barcelona, Spain). Functional images included 266 volumes for each individual and a gradient echo-planar imaging sequence depicting the blood oxygen level-dependent (BOLD) signal. Each volume contained 16 axial planes acquired with the following parameters: repetition time = 2000 ms, echo time = 20 ms, flip angle = 70°, section thickness = 7 mm, section skip = 0.7 mm, in-plane resolution = 3 × 3 mm. The first 10 volumes were discarded to avoid T1-saturation effects.

4.4. Statistical Analyses

4.4.1. Design

First, family-based genetic association analyses were conducted within EO families and AO families separately. Second, case–control genetic association analyses were tested by comparing HS to EO and AO patients independently. Third, neuroimaging analyses were developed to determine whether brain activity differences exist between HS and subjects with EO and AO SZ depending on the *NRN1* genetic variability.

4.4.2. Genetic Association Analyses

Haploview v4.1 [85] was employed to estimate the linkage disequilibrium (LD) between *NRN1* SNPs. Three haplotype blocks were identified (Block 1: SNP1-SNP3, Block 2: SNP4-SNP5 and Block 3: SNP6-SNP11) in both the family-based and the case–control sample (Supplementary Figure S5). Hardy–Weinberg and genetic association analyses between *NRN1* SNPs/haplotypes and SZ risk/age at onset were conducted using PLINK-v1.07 software [86].

For the family-based analyses, SNP and haplotype associations were tested using the Transmission Disequilibrium Test (TDT). This test evaluates whether the transmission frequency of alleles/haplotypes from heterozygous parents to their affected children deviates from the expected Mendelian frequency by comparing the transmitted and not transmitted alleles/haplotypes.

The case–control analyses were conducted using logistic regressions under different models of inheritance (allelic, genotypic/additive, recessive and dominant), all adjusted by sex.

In both genetic association approaches, a cut-off threshold for rare haplotypes of 1% and a sliding window approach were applied to the haplotype analyses. The odds ratios (OR) were estimated either from the absolute number of alleles/haplotypes transmitted and not transmitted from parents to affected offspring or from the absolute number of alleles/haplotypes estimated in patients and controls. Multiple testing corrections (10,000 permutations procedure) were applied to all analyses, and all the reported *p*-values are those obtained with this correction (p_{perm}). As haplotype TDT implemented in PLINK does not include the permutation procedure, to confirm our associations, all the possible haplotypes were reconstructed based on the most likely expectation maximisation (EM) phase and once reconstructed, haplotypic associations were tested using a simple TDT with 10,000 permutations.

The statistical power was calculated, in the case of the family-based sample, using the 'trio' R package version 3.1.2 available at http://www.bioconductor.org, accessed on 1 April 2022. As the sample consisted of 151 trios and the MAF of selected SNPs ranged from 0.20 to 0.50 (Table 1), by assuming an allelic TDT model and a statistical power of 0.80, the smaller detectable relative risk is 1.75. In the case of the case–control sample, the statistical power was calculated using the 'genpwr' R package version 1.0.2. As the sample

comprised 345 subjects and the MAF of selected SNPs ranged from 0.16 to 0.49, assuming a logistic model and a power of 0.80, the smaller detectable odds ratio is 1.57.

4.4.3. Neuroimaging Association Study

Based on our genetic association results highlighting the genetic region spanning SNP 6, 7 and 8, we performed the neuroimaging analyses with the haplotype significantly associated with the risk for EO (the HAP678 GTT) in the matched sub-set (39 HS, 39 EO and 39 AO subjects). For these analyses, individuals' possible haplotype phases were estimated using PLINK and only those with a probability $\geq$ 95% were included. Because of the haplotypic frequencies in our sample, the analyses were conducted considering the haplotype as a dichotomous variable, and each subject was classified as a non-carrier (0 copies of the risk haplotype) or carrier (1 or 2 copies of the risk haplotype).

Functional MRI pre-processing and analyses were performed with the FEAT tool, from FSL software (FMRIB Software Library, University of Oxford, Oxford, UK; [84]). Pre-processing included motion correction (using the MCFLIRT algorithm with 6 degrees of freedom) and co-registration and normalisation to a common stereotactic space (Montreal Neurological Institute [MNI] template with 2 $\times$ 2 $\times$ 2 mm resolution) using linear transformations with 12 degrees of freedom. Before group analyses, normalised images were spatially filtered with a Gaussian filter (FWHM = 5mm). To minimise unwanted movement-related effects, individuals with an estimated maximum absolute movement > 3.0 mm or an average absolute movement > 0.3 mm were excluded from analyses.

At the single-subject level analysis, General Linear Models (GLMs) were fitted to generate individual activation maps for each condition of interest compared to baseline and for the comparison between conditions (1-back vs. baseline, 2-back vs. baseline and 2-back vs. 1-back). Temporal derivatives for each condition of interest, as well as movement parameters (six in total, three rotations and three translations), were also included as additional regressors. Fixation periods were not modelled and thus acted as an implicit baseline (i.e., to compare a condition of interest of any given task with its baseline periods, the average BOLD signal from all the baseline periods across the whole task is subtracted from that of the blocks corresponding to the condition of interest). Images were high-pass filtered with a 130 s cut-off. All statistical tests were performed at the cluster level with a corrected p-value of 0.05 and an initial height threshold of 3.1 (equivalent to an uncorrected p-value of 0.001), using the Standard Field Theory correction implemented in FSL [87].

At the group-level analyses, we studied brain activations and deactivations associated with the execution of the N-back task within each group for all the contrasts, as well as the differences between groups using ANOVA models (two comparisons whole-brain corrected: HS vs. EO/HS vs. AO adjusted for age, sex, and premorbid-IQ).

Since the 1-back requires the maintenance of the target in the memory (keeping track of the target when the consecutive letter is represented) and the 2-back demands both maintenance and target switching (updating the target identity with the appearance of each new letter), we decided to focus our interaction analyses on the 2-back vs. 1-back contrast as it highlights regional responses specific to a higher WM capacity [88,89]. Accordingly, our group differences between HS and patients (both EO and AO), were more pronounced in the 2-back vs. 1-back contrast (Supplementary Figure S5).

The interaction effect between the diagnosis and the risk haplotype HAP678 GTT was investigated using a regression model, which tests whether the slope between groups and haplotype differs (two models, whole-brain corrected: HS and EO/HS and AO, adjusted for age, sex, and premorbid-IQ). Four contrasts were explored (EO > HS and the reverse contrast; AO > HS and the reverse contrast). Therefore, to control for all the comparisons, the significance threshold used was set to $p < 0.05/4 = 0.0125$. To interpret the direction of the interaction results, we estimated individual mean activity scores from the areas where a significant interaction was detected using the FSLSTATS tool in FSL and afterwards, these values were plotted using SPSS.

Analyses of the behavioural data (d'1 and d'2) were carried out using SPSS and ANOVA models adjusted for age, sex and estimated IQ. First, N-back task performance was compared between HS and patients (two comparisons: HS vs. EO/HS vs. AO). Second, we explored the effect of the risk haplotype HAP678 GTT on N-back task performance in each group (HS, EO and AO). Third, we tested the interaction between diagnosis and the risk haplotype HAP678 GTT (two models: HS vs. EO/HS vs. AO). Additionally, the change between d'1 and d'2 was explored using repeated measures ANOVA models adjusted for age, sex and estimated IQ. These repeated measures models were conducted, first, between groups (two comparisons: HS vs. EO/HS vs. AO) and, second, between groups depending on the haplotype (two models: HS vs. EO/HS vs. AO).

5. Conclusions

Our results contribute to the understanding of the molecular mechanisms underlying SZ early age at onset. Specifically, our work suggests that studying the role of neurotrophins (such as *NRN1*), in specific phenotypes with particularly etiological underpinnings (such as early-onset) and their effect on intermediate phenotypes (such as functional neuroimaging data), helps to elucidate the impact of common genetic variability on biological networks underlying mental disorders.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms23137456/s1.

Author Contributions: Conceptualisation, C.A.-P., C.G. and M.F.-V.; Data curation, C.A.-P., M.G.-R. and P.S.-P.; Formal analysis, C.A.-P., M.G.-R. and M.F.-V.; Funding acquisition, C.G., L.F., L.F.C., E.P.-C. and M.F.-V.; Investigation, C.A.-P., M.G.-R., M.G.-L., B.A. and M.F.-V.; Methodology, C.A.-P., R.S., A.G.-P., E.P.-C. and M.F.-V.; Project administration, M.F.-V.; Resources, M.G.-L., P.S.-P., S.M., M.J.M., L.L., M.P., M.I.C., M.J.C., T.M., S.S., E.P.-C. and M.F.-V.; Supervision, M.F.-V.; Visualisation, C.A.-P. and M.G.-R.; Writing—original draft, C.A.-P., M.G.-R. and M.F.-V.; Writing—review & editing, C.A.-P., M.G.-R., M.G.-L., C.G., P.S.-P., S.M., R.S., M.J.M., L.L., A.G.-P., M.P., M.I.C., M.J.C., T.M., S.S., L.F., L.F.C., B.A., E.P.-C. and M.F.-V. All authors have read and agreed to the published version of the manuscript.

Funding: This study received funding provided by: (i) Fundación Alicia Koplowitz; (ii) Acadèmia de les Ciències Mèdiques i de la Salut de Catalunya i de Balears (predoctoral contract to C.A.-P.); (iii) the Instituto de Salud Carlos III through a PFIS predoctoral contract to M.G.-R. (FI19/0352) and a Miguel Servet contract to M.F.-V. (CP20/00072), co-funded by European Regional Development Fund (ERDF)/European Social Fund "Investing in your future"; (iv) the Comissionat per a Universitats i Recerca del DIUE of the Generalitat de Catalunya (Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR), 2017SGR1271 and 2017SGR1577).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and ethical approval was obtained from the research ethical committee "CEIC Hermanas Hospitalarias del Sagrado Corazón de Jesús" (protocol code PR-2016-07 and date of approval 31 March 2016).

Informed Consent Statement: Informed consent statement was obtained from all the participants involved in the study. Written informed consent has been obtained from all the participants to publish this paper.

Conflicts of Interest: The authors declare to have no competing interest to declare.

References

1. Hilker, R.; Helenius, D.; Fagerlund, B.; Skytthe, A.; Christensen, K.; Werge, T.M.; Nordentoft, M.; Glenthøj, B. Heritability of schizophrenia and schizophrenia spectrum based on the nationwide Danish Twin register. *Biol. Psychiatry* **2018**, *83*, 492–498. [CrossRef] [PubMed]
2. Sullivan, P.F.; Kendler, K.S.; Neale, M.C. Schizophrenia as a complex trait: Evidence from a meta-analysis of twin studies. *Arch. Gen. Psychiatry* **2003**, *60*, 1187. [CrossRef] [PubMed]

3. Trubetskoy, V.; Pardiñas, A.F.; Qi, T.; Panagiotaropoulou, G.; Awasthi, S.; Bigdeli, T.B.; Bryois, J.; Chen, C.-Y.; Dennison, C.A.; Hall, L.S.; et al. Mapping genomic Loci implicates genes and synaptic biology in schizophrenia. *Nature* **2022**, *604*, 502–508. [CrossRef] [PubMed]
4. Pardiñas, A.F.; Holmans, P.; Pocklington, A.J.; Escott-Price, V.; Ripke, S.; Carrera, N.; Legge, S.E.; Bishop, S.; Cameron, D.; Hamshere, M.L.; et al. Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection. *Nat. Genet.* **2018**, *50*, 381–389. [CrossRef] [PubMed]
5. Ripke, S.; Neale, B.M.; Corvin, A.; Walters, J.T.R. Biological insights from 108 schizophrenia-associated genetic Loci. *Nature* **2014**, *511*, 421–427. [CrossRef]
6. Liang, S.G.; Greenwood, T.A. The impact of clinical heterogeneity in schizophrenia on genomic analyses. *Schizophr. Res.* **2015**, *161*, 490–495. [CrossRef]
7. Glahn, D.C.; Nimgaonkar, V.L.; Raventós, H.; Contreras, J.; McIntosh, A.M.; Thomson, P.A.; Jablensky, A.; McCarthy, N.S.; Charlesworth, J.C.; Blackburn, N.B.; et al. Rediscovering the value of families for psychiatric genetics research. *Mol. Psychiatry* **2019**, *24*, 523–535. [CrossRef]
8. Lange, E.M.; Sun, J.; Lange, L.A.; Zheng, S.L.; Duggan, D.; Carpten, J.D.; Gronberg, H.; Isaacs, W.B.; Xu, J.; Chang, B.-L. Family-based samples can play an important role in genetic association studies. *Cancer Epidemiol. Prev. Biomark.* **2008**, *17*, 2208–2214. [CrossRef]
9. Hare, E.; Glahn, D.C.; Dassori, A.; Raventos, H.; Nicolini, H.; Ontiveros, A.; Medina, R.; Mendoza, R.; Jerez, A.; Muñoz, R.; et al. Heritability of age of onset of psychosis in schizophrenia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **2010**, *153*, 298–302. [CrossRef]
10. Esterberg, M.L.; Trotman, H.D.; Holtzman, C.; Compton, M.T.; Walker, E.F. The impact of a family history of psychosis on age-at-onset and positive and negative symptoms of schizophrenia: A meta-analysis. *Schizophr. Res.* **2010**, *120*, 121–130. [CrossRef]
11. Hilker, R.; Helenius, D.; Fagerlund, B.; Skytthe, A.; Christensen, K.; Werge, T.M.; Nordentoft, M.; Glenthøj, B. Is an early age at illness onset in schizophrenia associated with increased genetic susceptibility? Analysis of data from the nationwide Danish Twin register. *EBioMedicine* **2017**, *18*, 320–326. [CrossRef] [PubMed]
12. Hollis, C. Adult outcomes of child- and adolescent-onset schizophrenia: Diagnostic stability and predictive validity. *Am. J. Psychiatry* **2000**, *157*, 1652–1659. [CrossRef] [PubMed]
13. Forsyth, J.K.; Asarnow, R.F. Genetics of childhood-onset schizophrenia: 2019 update. *Child Adolesc. Psychiatr. Clin. N. Am.* **2020**, *29*, 157–170. [CrossRef] [PubMed]
14. Larsen, T.K.; Friis, S.; Haahr, U.; Johannessen, J.O.; Melle, I.; Opjordsmoen, S.; Rund, B.R.; Simonsen, E.; Vaglum, P.; McGlashan, T.H. Premorbid adjustment in first-episode non-affective psychosis: Distinct patterns of pre-onset course. *Br. J. Psychiatry* **2004**, *185*, 108–115. [CrossRef] [PubMed]
15. Rajji, T.K.; Ismail, Z.; Mulsant, B.H. Age at onset and cognition in schizophrenia: Meta-analysis. *Br. J. Psychiatry* **2009**, *195*, 286–293. [CrossRef]
16. Clemmensen, L.; Vernal, D.L.; Steinhausen, H.-C. A systematic review of the long-term outcome of early onset schizophrenia. *BMC Psychiatry* **2012**, *12*, 150. [CrossRef]
17. Rapoport, J.L.; Addington, A.M.; Frangou, S.; Psych, M.R.C. The neurodevelopmental model of schizophrenia: Update. *Mol. Psychiatry* **2005**, *10*, 434–449. [CrossRef]
18. Rapoport, J.L.; Gogtay, N. Childhood onset schizophrenia: Support for a progressive neurodevelopmental disorder. *Int. J. Dev. Neurosci. Off. J. Int. Soc. Dev. Neurosci.* **2011**, *29*, 251–258. [CrossRef]
19. Bergen, S.E.; O'Dushlaine, C.T.; Lee, P.H.; Fanous, A.H.; Ruderfer, D.M.; Ripke, S.; Sullivan, P.F.; Smoller, J.W.; Purcell, S.M.; Corvin, A. Genetic modifiers and subtypes in schizophrenia: Investigations of age at onset, severity, sex and family history. *Schizophr. Res.* **2014**, *154*, 48–53. [CrossRef]
20. Guo, S.; Liu, J.; Li, W.; Yang, Y.; Lv, L.; Xiao, X.; Li, M.; Guan, F.; Luo, X.-J. Genome wide association study identifies four loci for early onset schizophrenia. *Transl. Psychiatry* **2021**, *11*, 248. [CrossRef]
21. Wang, K.-S.; Liu, X.; Zhang, Q.; Aragam, N.; Pan, Y. Genome-wide association analysis of age at onset in schizophrenia in a European-American sample. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **2011**, *156*, 671–680. [CrossRef] [PubMed]
22. Woolston, A.L.; Hsiao, P.-C.; Kuo, P.-H.; Wang, S.-H.; Lien, Y.-J.; Liu, C.-M.; Hwu, H.-G.; Lu, T.-P.; Chuang, E.Y.; Chang, L.-C.; et al. Genetic Loci associated with an earlier age at onset in multiplex schizophrenia. *Sci. Rep.* **2017**, *7*, 1–9. [CrossRef] [PubMed]
23. Ahn, K.; An, S.S.; Shugart, Y.Y.; Rapoport, J.L. Common polygenic variation and risk for childhood-onset schizophrenia. *Mol. Psychiatry* **2016**, *21*, 94–96. [CrossRef] [PubMed]
24. Zhou, S.; Zhou, J. Neuritin, a Neurotrophic factor in nervous system physiology. *Curr. Med. Chem.* **2014**, *21*, 1212–1219. [CrossRef]
25. Aid, T.; Kazantseva, A.; Piirsoo, M.; Palm, K.; Timmusk, T. Mouse and rat BDNF gene structure and expression revisited. *J. Neurosci. Res.* **2007**, *85*, 525–535. [CrossRef]
26. Nedivi, E.; Fieldust, S.; Theill, L.E.; Hevron, D. A Set of genes expressed in response to light in the adult cerebral cortex and regulated during development. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 2048–2053. [CrossRef]
27. Nedivi, E.; Hevroni, D.; Naot, D.; Israeli, D.; Citri, Y. Numerous candidate plasticity-related genes revealed by differential cDNA cloning. *Nature* **1993**, *363*, 718–722. [CrossRef]
28. Tongiorgi, E. Activity-Dependent expression of brain-derived neurotrophic factor in dendrites: Facts and open questions. *Neurosci. Res.* **2008**, *61*, 335–346. [CrossRef]

29. Lu, J.-M.; Liu, D.-D.; Li, Z.-Y.; Ling, C.; Mei, Y.-A. Neuritin enhances synaptic transmission in medial prefrontal cortex in mice by increasing CaV3.3 surface expression. *Cereb. Cortex* **2017**, *27*, 3842–3855. [CrossRef]
30. Yao, J.-J.; Gao, X.-F.; Chow, C.-W.; Zhan, X.-Q.; Hu, C.-L.; Mei, Y.-A. Neuritin activates insulin receptor pathway to up-regulate Kv4.2-mediated transient outward K^+ current in rat cerebellar granule neurons. *J. Biol. Chem.* **2012**, *287*, 41534–41545. [CrossRef]
31. An, K.; Jung, J.H.; Jeong, A.Y.; Kim, H.G.; Jung, S.Y.; Lee, K.; Kim, H.J.; Kim, S.-J.; Jeong, T.-Y.; Son, Y.; et al. Neuritin can normalize neural deficits of Alzheimer's disease. *Cell Death Dis.* **2014**, *5*, e1523. [CrossRef] [PubMed]
32. Son, H.; Banasr, M.; Choi, M.; Chae, S.Y.; Licznerski, P.; Lee, B.; Voleti, B.; Li, N.; Lepack, A.; Fournier, N.M.; et al. Neuritin produces antidepressant actions and blocks the neuronal and behavioral deficits caused by chronic stress. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 11378–11383. [CrossRef] [PubMed]
33. Choi, Y.; Lee, K.; Ryu, J.; Kim, H.G.; Jeong, A.Y.; Woo, R.-S.; Lee, J.-H.; Hyun, J.W.; Hahn, S.; Kim, J.-H.; et al. Neuritin attenuates cognitive function impairments in Tg2576 mouse model of Alzheimer's Disease. *PLoS ONE* **2014**, *9*, e104121. [CrossRef]
34. Park, K.W.; Kim, I.H.; Sun, W.; Kim, H. Sustained expression of neuritin MRNA after repeated electroconvulsive stimulations in the rat hippocampal formation. *Exp. Neurobiol.* **2009**, *18*, 62. [CrossRef]
35. Chandler, D.; Dragović, M.; Cooper, M.; Badcock, J.C.; Mullin, B.H.; Faulkner, D.; Wilson, S.G.; Hallmayer, J.; Howell, S.; Rock, D.; et al. Impact of neuritin 1 (*NRN1*) polymorphisms on fluid intelligence in schizophrenia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **2010**, *153*, 428–437. [CrossRef]
36. Fatjó-Vilas, M.; Prats, C.; Pomarol-Clotet, E.; Lázaro, L.; Moreno, C.; González-Ortega, I.; Lera-Miguel, S.; Miret, S.; Muñoz, M.J.; Ibáñez, I.; et al. Involvement of NRN1 gene in schizophrenia-spectrum and bipolar disorders and its impact on age at onset and cognitive functioning. *World J. Biol. Psychiatry* **2016**, *17*, 129–139. [CrossRef]
37. Birnbaum, R.; Weinberger, D.R. Functional neuroimaging and schizophrenia: A view towards effective connectivity modeling and polygenic risk. *Dialogues Clin. Neurosci.* **2013**, *15*, 279–289. [CrossRef]
38. Bora, E.; Lin, A.; Wood, S.J.; Yung, A.R.; McGorry, P.D.; Pantelis, C. Cognitive deficits in youth with familial and clinical high risk to psychosis: A systematic review and meta-analysis. *Acta Psychiatr. Scand.* **2014**, *130*, 1–15. [CrossRef]
39. Zhang, R.; Picchioni, M.; Allen, P.; Toulopoulou, T. Working memory in unaffected relatives of patients with schizophrenia: A meta-analysis of functional magnetic resonance imaging studies. *Schizophr. Bull.* **2016**, *42*, 1068–1077. [CrossRef]
40. Repovš, G.; Barch, D.M. Working memory related brain network connectivity in individuals with schizophrenia and their siblings. *Front. Hum. Neurosci.* **2012**, *6*, 137. [CrossRef]
41. Silver, H.; Feldman, P.; Bilker, W.; Gur, R.C. Working memory deficit as a core neuropsychological dysfunction in schizophrenia. *Am. J. Psychiatry* **2003**, *160*, 1809–1816. [CrossRef] [PubMed]
42. Van Erp, T.G.M.; Walton, E.; Hibar, D.P.; Schmaal, L.; Jiang, W.; Glahn, D.C.; Pearlson, G.D.; Yao, N.; Fukunaga, M.; Hashimoto, R.; et al. Cortical brain abnormalities in 4474 individuals with schizophrenia and 5098 control subjects via the enhancing neuro imaging genetics through meta analysis (ENIGMA) consortium. *Biol. Psychiatry* **2018**, *84*, 644–654. [CrossRef] [PubMed]
43. Bittner, R.A.; Linden, D.E.J.; Roebroeck, A.; Härtling, F.; Rotarska-Jagiela, A.; Maurer, K.; Goebel, R.; Singer, W.; Haenschel, C. The when and where of working memory dysfunction in early-onset schizophrenia—A functional magnetic resonance imaging study. *Cereb. Cortex* **2015**, *25*, 2494–2506. [CrossRef] [PubMed]
44. Kyriakopoulos, M.; Dima, D.; Roiser, J.P.; Corrigall, R.; Barker, G.J.; Frangou, S. Abnormal functional activation and connectivity in the working memory network in early-onset schizophrenia. *J. Am. Acad. Child Adolesc. Psychiatry* **2012**, *51*, 911–920.e2. [CrossRef] [PubMed]
45. Loeb, F.F.; Zhou, X.; Craddock, K.E.S.; Shora, L.; Broadnax, D.D.; Gochman, P.; Clasen, L.S.; Lalonde, F.M.; Berman, R.A.; Berman, K.F.; et al. Reduced functional brain activation and connectivity during a working memory task in childhood-onset schizophrenia. *J. Am. Acad. Child Adolesc. Psychiatry* **2018**, *57*, 166–174. [CrossRef] [PubMed]
46. Pauly, K.; Seiferth, N.Y.; Kellermann, T.; Backes, V.; Vloet, T.D.; Shah, N.J.; Schneider, F.; Habel, U.; Kircher, T.T. Cerebral dysfunctions of emotion—Cognition interactions in adolescent-onset schizophrenia. *J. Am. Acad. Child Adolesc. Psychiatry* **2008**, *47*, 1299–1310. [CrossRef] [PubMed]
47. Thormodsen, R.; Jensen, J.; Holmèn, A.; Juuhl-Langseth, M.; Emblem, K.E.; Andreassen, O.A.; Rund, B.R. Prefrontal hyperactivation during a working memory task in early-onset schizophrenia spectrum disorders: An FMRI study. *Psychiatry Res. Neuroimaging* **2011**, *194*, 257–262. [CrossRef]
48. White, T.; Hongwanishkul, D.; Schmidt, M. Increased anterior cingulate and temporal lobe activity during visuospatial working memory in children and adolescents with schizophrenia. *Schizophr. Res.* **2011**, *125*, 118–128. [CrossRef]
49. Solmi, M.; Radua, J.; Olivola, M.; Croce, E.; Soardo, L.; Salazar de Pablo, G.; Il Shin, J.; Kirkbride, J.B.; Jones, P.; Kim, J.H.; et al. Age at onset of mental disorders worldwide: Large-scale meta-analysis of 192 epidemiological studies. *Mol. Psychiatry* **2021**, *27*, 1–15. [CrossRef]
50. Lindholm, E.; Ekholm, B.; Shaw, S.; Jalonen, P.; Johansson, G.; Pettersson, U.; Sherrington, R.; Adolfsson, R.; Jazin, E. A schizophrenia-susceptibility locus at 6q25, in one of the world's largest reported pedigrees. *Am. J. Hum. Genet.* **2001**, *69*, 96–105. [CrossRef]
51. Maziade, M.; Roy, M.-A.; Rouillard, É.; Bissonnette, L.; Fournier, J.-P.; Roy, A.; Garneau, Y.; Montgrain, N.; Potvin, A.; Cliche, D.; et al. A search for specific and common susceptibility loci for schizophrenia and bipolar disorder: A linkage study in 13 target chromosomes. *Mol. Psychiatry* **2001**, *6*, 684–693. [CrossRef] [PubMed]

52. Moises, H.W.; Yang, L.; Kristbjarnarson, H.; Wiese, C.; Byerley, W.; Macciardi, F.; Arolt, V.; Blackwood, D.; Liu, X.; Sjögren, B.; et al. An international two–stage genome–wide search for schizophrenia susceptibility genes. *Nat. Genet.* **1995**, *11*, 321–324. [CrossRef] [PubMed]
53. Straub, R.E.; MacLean, C.J.; Ma, Y.; Webb, B.T.; Myakishev, M.V.; Harris-Kerr, C.; Wormley, B.; Sadek, H.; Kadambi, B.; O'Neill, F.A.; et al. Genome-wide scans of three independent sets of 90 Irish Multiplex schizophrenia families and follow-up of selected regions in all families provides evidence for multiple susceptibility genes. *Mol. Psychiatry* **2002**, *7*, 542–559. [CrossRef] [PubMed]
54. Fanous, A.H.; Neale, M.C.; Webb, B.T.; Straub, R.E.; Amdur, R.L.; O'Neill, F.A.; Walsh, D.; Riley, B.P.; Kendler, K.S. A genome-wide scan for modifier Loci in schizophrenia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **2007**, *144*, 589–595. [CrossRef]
55. Pidsley, R.; Viana, J.; Hannon, E.; Spiers, H.; Troakes, C.; Al-Saraj, S.; Mechawar, N.; Turecki, G.; Schalkwyk, L.C.; Bray, N.J.; et al. Methylomic profiling of human brain tissue supports a neurodevelopmental origin for schizophrenia. *Genome Biol.* **2014**, *15*, 483. [CrossRef]
56. Bähner, F.; Meyer-Lindenberg, A. Hippocampal–prefrontal connectivity as a translational phenotype for schizophrenia. *Eur. Neuropsychopharmacol.* **2017**, *27*, 93–106. [CrossRef]
57. Riley, M.R.; Constantinidis, C. Role of prefrontal persistent activity in working memory. *Front. Syst. Neurosci.* **2016**, *9*, 181. [CrossRef]
58. Thomas, M.L.; Duffy, J.R.; Swerdlow, N.; Light, G.A.; Brown, G.G. Detecting the inverted-U in FMRI studies of schizophrenia: A comparison of three analysis methods. *J. Int. Neuropsychol. Soc.* **2022**, *28*, 258–269. [CrossRef]
59. Prats, C.; Arias, B.; Ortet, G.; Ibáñez, M.I.; Moya, J.; Pomarol-Clotet, E.; Fañanás, L.; Fatjó-Vilas, M. Role of neurotrophins in depressive symptoms and executive function: Association analysis of NRN1 gene and its interaction with BDNF gene in a non-clinical sample. *J. Affect. Disord.* **2017**, *211*, 92–98. [CrossRef]
60. Lenroot, R.K.; Giedd, J.N. Brain development in children and adolescents: Insights from anatomical magnetic resonance imaging. *Neurosci. Biobehav. Rev.* **2006**, *30*, 718–729. [CrossRef]
61. Fair, D.A.; Dosenbach, N.U.F.; Church, J.A.; Cohen, A.L.; Brahmbhatt, S.; Miezin, F.M.; Barch, D.M.; Raichle, M.E.; Petersen, S.E.; Schlaggar, B.L. Development of distinct control networks through segregation and integration. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 13507–13512. [CrossRef] [PubMed]
62. Finn, A.S.; Sheridan, M.A.; Kam, C.L.H.; Hinshaw, S.; D'Esposito, M. Longitudinal evidence for functional specialization of the neural circuit supporting working memory in the human brain. *J. Neurosci.* **2010**, *30*, 11062–11067. [CrossRef] [PubMed]
63. Andre, J.; Picchioni, M.; Zhang, R.; Toulopoulou, T. Working memory circuit as a function of increasing age in healthy adolescence: A systematic review and meta-analyses. *NeuroImage Clin.* **2015**, *12*, 940–948. [CrossRef] [PubMed]
64. Hartshorne, J.K.; Germine, L.T. When does cognitive functioning peak? The asynchronous rise and fall of different cognitive abilities across the lifespan. *Psychol. Sci.* **2015**, *26*, 433–443. [CrossRef] [PubMed]
65. Bowen, E.F.W.; Burgess, J.L.; Granger, R.; Kleinman, J.E.; Rhodes, C.H. DLPFC Transcriptome defines two molecular subtypes of schizophrenia. *Transl. Psychiatry* **2019**, *9*, 1–10. [CrossRef]
66. Singh-Taylor, A.; Molet, J.; Jiang, S.; Korosi, A.; Bolton, J.L.; Noam, Y.; Simeone, K.; Cope, J.; Chen, Y.; Mortazavi, A.; et al. NRSF-Dependent epigenetic mechanisms contribute to programming of stress-sensitive neurons by neonatal experience, promoting resilience. *Mol. Psychiatry* **2018**, *23*, 648–657. [CrossRef]
67. Ohi, K.; Hashimoto, R.; Yasuda, Y.; Kiribayashi, M.; Iike, N.; Yoshida, T.; Azechi, M.; Ikezawa, K.; Takahashi, H.; Morihara, T.; et al. TATA box-binding protein gene is associated with risk for schizophrenia, age at onset and prefrontal function. *Genes Brain Behav.* **2009**, *8*, 473–480. [CrossRef]
68. Zhang, J.; Chang, L.; Pu, Y.; Hashimoto, K. Abnormal expression of colony stimulating factor 1 receptor (CSF1R) and transcription factor PU.1 (SPI1) in the spleen from patients with major psychiatric disorders: A role of brain–spleen axis. *J. Affect. Disord.* **2020**, *272*, 110–115. [CrossRef]
69. Lee, S.H.; DeCandia, T.R.; Ripke, S.; Yang, J.; Sullivan, P.F.; Goddard, M.E.; Keller, M.C.; Visscher, P.M.; Wray, N.R. Estimating the proportion of variation in susceptibility to schizophrenia captured by common SNPs. *Nat. Genet.* **2012**, *44*, 247–250. [CrossRef]
70. Singh, T.; Poterba, T.; Curtis, D.; Akil, H.; Al Eissa, M.; Barchas, J.D.; Bass, N.; Bigdeli, T.B.; Breen, G.; Bromet, E.J.; et al. Rare coding variants in ten genes confer substantial risk for schizophrenia. *Nature* **2022**, *604*, 509–516. [CrossRef]
71. Fusar-Poli, P. Voxel-wise meta-analysis of FMRI studies in patients at clinical high risk for psychosis. *J. Psychiatry Neurosci.* **2012**, *37*, 106–112. [CrossRef] [PubMed]
72. Radua, J.; Borgwardt, S.; Crescini, A.; Mataix-Cols, D.; Meyer-Lindenberg, A.; McGuire, P.K.; Fusar-Poli, P. Multimodal meta-analysis of structural and functional brain changes in first episode psychosis and the effects of antipsychotic medication. *Neurosci. Biobehav. Rev.* **2012**, *36*, 2325–2333. [CrossRef] [PubMed]
73. Geller, B.; Zimerman, B.; Williams, M.; Bolhofner, K.; Craney, J.L.; Delbello, M.P.; Soutullo, C. Reliability of the Washington University in St. Louis kiddie schedule for affective disorders and schizophrenia (WASH-U-KSADS) mania and rapid cycling sections. *J. Am. Acad. Child Adolesc. Psychiatry* **2001**, *40*, 450–455. [CrossRef]
74. Andreasen, N.C. The comprehensive assessment of symptoms and history (CASH): An instrument for assessing diagnosis and psychopathology. *Arch. Gen. Psychiatry* **1992**, *49*, 615. [CrossRef]
75. First, M.; Spitzer, R.; Gibbon, M.; Williams, J. Structured clinical interview for DSM-IV-TR Axis I disorders, research version, non-patient edition. In *(SCID-I/P)*; New York State Psychiatric Institute: New York, NY, USA, 2002.

76. Perkins, D.O.; Leserman, J.; Jarskog, L.F.; Graham, K.; Kazmer, J.; Lieberman, J.A. Characterizing and dating the onset of symptoms in psychotic illness: The symptom onset in schizophrenia (SOS) inventory. *Schizophr. Res.* **2000**, *44*, 1–10. [CrossRef]
77. Gershon, E.S.; DeLisi, L.E.; Hamovit, J.; Nurnberger, J.I., Jr.; Maxwell, M.E.; Schreiber, J.; Dauphinais, D.; Dingman, C.W., II; Guroff, J.J. A controlled family study of chronic psychoses: Schizophrenia and schizoaffective disorder. *Arch. Gen. Psychiatry* **1988**, *45*, 328–336. [CrossRef] [PubMed]
78. Gomar, J.J.; Ortiz-Gil, J.; McKenna, P.J.; Salvador, R.; Sans-Sansa, B.; Sarró, S.; Guerrero, A.; Pomarol-Clotet, E. Validation of the word accentuation test (TAP) as a means of estimating premorbid IQ in spanish speakers. *Schizophr. Res.* **2011**, *128*, 175–176. [CrossRef]
79. Zhang, F.; Lupski, J.R. Non-coding genetic variants in human disease. *Hum. Mol. Genet.* **2015**, *24*, R102–R110. [CrossRef]
80. Fullard, J.F.; Giambartolomei, C.; Hauberg, M.E.; Xu, K.; Voloudakis, G.; Shao, Z.; Bare, C.; Dudley, J.T.; Mattheisen, M.; Robakis, N.K.; et al. Open chromatin profiling of human postmortem brain infers functional roles for non-coding schizophrenia Loci. *Hum. Mol. Genet.* **2017**, *26*, 1942–1951. [CrossRef]
81. Ward, L.D.; Kellis, M. HaploReg: A resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res.* **2012**, *40*, D930–D934. [CrossRef]
82. Boyle, E.A.; Li, Y.I.; Pritchard, J.K. An expanded view of complex traits: From polygenic to omnigenic. *Cell* **2017**, *169*, 1177–1186. [CrossRef] [PubMed]
83. Dong, S.; Boyle, A.P. Predicting functional variants in enhancer and promoter elements using regulomeDB. *Hum. Mutat.* **2019**, *40*, 1292–1298. [CrossRef] [PubMed]
84. Smith, S.M.; Jenkinson, M.; Woolrich, M.W.; Beckmann, C.F.; Behrens, T.E.J.; Johansen-Berg, H.; Bannister, P.R.; De Luca, M.; Drobnjak, I.; Flitney, D.E.; et al. Advances in functional and structural mr image analysis and implementation as FSL. *NeuroImage* **2004**, *23*, S208–S219. [CrossRef]
85. Barrett, J.C.; Fry, B.; Maller, J.; Daly, M.J. Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics* **2005**, *21*, 263–265. [CrossRef] [PubMed]
86. Purcell, S.; Neale, B.; Todd-Brown, K.; Thomas, L.; Ferreira, M.A.R.; Bender, D.; Maller, J.; Sklar, P.; de Bakker, P.I.W.; Daly, M.J.; et al. PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **2007**, *81*, 559–575. [CrossRef] [PubMed]
87. Worsley, K.J. Statistical Analysis of Activation Images. In *Functional Magnetic Resonance Imaging*; Oxford University Press: Oxford, UK, 2001; ISBN 978-0-19-263071-1.
88. Egli, T.; Coynel, D.; Spalek, K.; Fastenrath, M.; Freytag, V.; Heck, A.; Loos, E.; Auschra, B.; Papassotiropoulos, A.; de Quervain, D.J.-F.; et al. Identification of two distinct working memory-related brain networks in healthy young adults. *eNeuro* **2018**, *5*, ENEURO.0222-17.2018. [CrossRef]
89. Wilhelm, O.; Hildebrandt, A.; Oberauer, K. What is working memory capacity, and how can we measure it? *Front. Psychol.* **2013**, *4*, 433. [CrossRef]

Study I.II

NRN1 epistasis with *BDNF* and *CACNA1C*: mediation effects on symptom severity through neuroanatomical changes in schizophrenia

Almodóvar-Payá C, Guardiola-Ripoll M, Giralt-López M, Oscoz-Irurozqui M, Canales-Rodríguez EJ, Madre M, Soler-Vidal J, Ramiro N, Callado LF, Arias B, Gallego C, Pomarol-Clotet E, Fatjó-Vilas M

Brain Structure and Function. 2024 Jun 229(5):1299-1315.

doi: 10.1007/s00429-024-02793-5. PMID: 38720004

Brain Structure and Function (2024) 229:1299–1315
https://doi.org/10.1007/s00429-024-02793-5

ORIGINAL ARTICLE

NRN1 epistasis with *BDNF* and *CACNA1C*: mediation effects on symptom severity through neuroanatomical changes in schizophrenia

Carmen Almodóvar-Payá[1,2,3] · Maria Guardiola-Ripoll[1,15] · Maria Giralt-López[4,5] · Maitane Oscoz-Irurozqui[1,6] · Erick Jorge Canales-Rodríguez[1,3,7] · Mercè Madre[1,8] · Joan Soler-Vidal[1,3,9] · Núria Ramiro[10] · Luis F. Callado[3,11,12] · Bárbara Arias[2,3,13] · Carme Gallego[14] · Edith Pomarol-Clotet[1,3] · Mar Fatjó-Vilas[1,2,3]

Received: 27 December 2023 / Accepted: 19 March 2024 / Published online: 9 May 2024

Abstract
The expression of Neuritin-1 (*NRN1*), a neurotrophic factor crucial for neurodevelopment and synaptic plasticity, is enhanced by the Brain Derived Neurotrophic Factor (*BDNF*). Although the receptor of NRN1 remains unclear, it is suggested that NRN1's activation of the insulin receptor (IR) pathway promotes the transcription of the calcium voltage-gated channel subunit alpha1 C (*CACNA1C*). These three genes have been independently associated with schizophrenia (SZ) risk, symptomatology, and brain differences. However, research on how they synergistically modulate these phenotypes is scarce. We aimed to study whether the genetic epistasis between these genes affects the risk and clinical presentation of the disorder via its effect on brain structure. First, we tested the epistatic effect of *NRN1* and *BDNF* or *CACNA1C* on (i) the risk for SZ, (ii) clinical symptoms severity and functionality (onset, PANSS, CGI and GAF), and (iii) brain cortical structure (thickness, surface area and volume measures estimated using FreeSurfer) in a sample of 86 SZ patients and 89 healthy subjects. Second, we explored whether those brain clusters influenced by epistatic effects mediate the clinical profiles. Although we did not find a direct epistatic impact on the risk, our data unveiled significant effects on the disorder's clinical presentation. Specifically, the *NRN1*-rs10484320 x *BDNF*-rs6265 interplay influenced PANSS general psychopathology, and the *NRN1*-rs4960155 x *CACNA1C*-rs1006737 interaction affected GAF scores. Moreover, several interactions between *NRN1* SNPs and *BDNF*-rs6265 significantly influenced the surface area and cortical volume of the frontal, parietal, and temporal brain regions within patients. The *NRN1*-rs10484320 x *BDNF*-rs6265 epistasis in the left lateral orbitofrontal cortex fully mediated the effect on PANSS general psychopathology. Our study not only adds clinical significance to the well-described molecular relationship between *NRN1* and *BDNF* but also underscores the utility of deconstructing SZ into biologically validated brain-imaging markers to explore their mediation role in the path from genetics to complex clinical manifestation.

Keywords Schizophrenia · Genetic epistasis · Symptoms · Neuroimaging · Mediation

Background

Schizophrenia (SZ) is a severe psychiatric disorder with a substantial burden affecting 21 million people worldwide (Charlson et al. 2018). It has a strong genetic component that is reflected in its high heritability ($h^2 = 65–79\%$) (Hilker et al. 2018; Sullivan et al. 2003). Moreover, genome-wide association studies (GWAS) have provided solid evidence of its polygenicity by describing a hundred genetic variants with additive effects (Pardiñas et al. 2018; Trubetskoy et al. 2022). Interestingly, due to the convergence of those variants in molecular networks related to synaptic plasticity, it has been proposed as a key pathophysiological mechanism in SZ (Hall and Bray 2022). However, the identified risk variants together only account for a proportion ($h^2_{SNP} = 24\%$) of the variance in liability of the phenotype (Trubetskoy et al. 2022).

It has been suggested that such *missing heritability* could arise, among other factors, from nonlinear molecular

Carmen Almodóvar-Payá and Maria Guardiola-Ripoll contributed equally to co-first authors.

Edith Pomarol-Clotet and Mar Fatjó-Vilas contributed equally to co-senior authors.

Extended author information available on the last page of the article

interactions, which are not typically explored in classical GWAS models (Zuk et al. 2012). In fact, gene-gene interactions between two or more loci have been extensively studied in animal and cellular models, suggesting that epistatic networks might represent an essential molecular mechanism involved in the modulation of complex traits (Mackay and Moore 2014; Özsoy et al. 2021). When the collective effect of large-scale genetic interactions is considered in humans, the association with complex traits, such as SZ, becomes more robust and new variants appear (Woo et al. 2017). Therefore, considering that genetic interactions mainly occur between genes involved in the same molecular pathways (Roguev et al. 2008), inspecting epistatic effects among those involved in synaptic plasticity in SZ may add relevant data on how genetic factors lead to the emergence of the disorder.

Considering the phenotypic complexity of SZ as reflected in its diverse outcomes, neuroimaging genetics approaches provide a neurobiological context for studying how genetic variants act to confer an increased risk for the disorder (van der Meer and Kaufmann 2022). Notably, SZ-related genes identified by GWAS are highly expressed in brain regions with structural differences in SZ (Ji et al. 2021) and have been associated with symptomatology (Legge et al. 2021; Sengupta et al. 2017). This indicates a partial genetic overlap within the axis connecting the brain and symptoms, with specific genes delineated as potential contributors to cerebral alterations that sustain distinct manifestations. Indeed, some studies have shown that genetic effects on clinical phenotypes are mediated by specific brain regions and functions in mental disorders (Sudre et al. 2020). Nevertheless, few cases in the literature explore the impact of epistasis on brain phenotypes (Callicott et al. 2013; Guardiola-Ripoll et al. 2022; Tecelão et al. 2019; Xu et al. 2018) and its mediation effect on clinical symptoms in SZ. These studies have highlighted the methodological challenges in exploring epistasis while effectively unveiling non-independent effects between those genes that go unnoticed when examining main effects alone. Therefore, such approaches reveal the important role of gene interactions in the architecture of common human diseases while helping to connect the statistical perspective with the complex dynamics of biological systems (Phillips 2008).

One of the genes intimately implicated in synaptic plasticity processes is Neuritin-1 (*NRN1*, 6p25.1), which encodes for a neurotrophin that is highly expressed in the hippocampus, the cerebral cortex and the cerebellum (Naeve et al. 1997). Its expression is experience-dependent (Harwell et al. 2005; Nedivi et al. 1996) and regulated by Ca^{2+} influx via the N-methyl-D-aspartate (NMDA) receptors (Fujino et al. 2003). Additionally, it has been described that another neuropeptide, the Brain-Derived Neurotrophic Factor (*BDNF*, 11p13), modulates *NRN1* expression. This gene is highly expressed in the same cerebral regions as *NRN1* (Esvald et al. 2023) and also in an experience-dependent manner (Tongiorgi 2008). As neurotrophins, both genes exert multiple functions in the developing brain, being involved, for example, in enhancing neurite and dendritic growth, stabilising active synapses, improving synaptic maturation, increasing neuronal migration and regulating apoptosis of proliferative neurons, but are also involved in regulating the neuronal plasticity in the adult brain (Sasi et al. 2017; Yao et al. 2018). In the synaptic cleft, BDNF binds tyrosine kinase receptor B (TrkB) activating the transcription factor CREB (cAMP response element-binding protein) that attaches, among others, to the endogenous *NRN1* promoter in vivo (Finkbeiner et al. 1997; Fujino et al. 2003) fostering functional and structural neuronal changes and leading to the consolidation of long-term synaptic plasticity (Kaldun and Sprecher 2019). Besides, animal-based models have described the direct relationship between *BDNF* and *NRN1* expression, showing that the intraventricular injection or the intrahippocampal infusion of BDNF into neonatal rat pups results in the up-regulation of *NRN1* expression in vivo (Wibrand et al. 2006).

Current research looking for potential receptors for *NRN1* suggests a role in regulating synaptic excitability through the activation of the insulin receptor (IR) and downstream pathway, which trigger the transcription and trafficking of L-type voltage-gated calcium channel (L-VGCC) subunits to the membrane of cortical neurons, specifically Cav1.3 and Cav1.2 (Lu et al. 2017; J.-J. Yao et al. 2012; Zhao et al. 2018). The latter is encoded by humans' calcium voltage-gated channel subunit alpha1 C gene (*CACNA1C*, 12p13.33). Notably, L-VGCC channels constitute the most abundant calcium channels in the human brain, accounting for approximately 90% (Striessnig et al. 2014). These channels are located at the post-synapsis, specifically at the neurons' soma and dendritic spines and shaft (Jenkins et al. 2010). There, they facilitate Ca^{2+} influx in response to membrane depolarisation, which acts as a cellular messenger that triggers diverse cellular responses, including the strengthening of short- and long-term synaptic plasticity and the promotion of activity-dependent gene expression in a process called excitation-transcription coupling (Ma et al. 2011).

The genetic association of *BDNF* and *CACNA1C* genes has been widely reported in psychiatric disorders, and *NRN1*, while far less explored, has been identified as an interesting candidate gene, involved in age at onset, general cognitive abilities and brain activity in SZ (Almodóvar-Payá et al. 2022; Chandler et al. 2010; Fatjó-Vilas et al. 2016). Regarding the role of *BDNF* in SZ, the rs6265 polymorphism has been associated not only with the risk for the disorder (Kheirollahi et al. 2016; Rosa et al. 2006) but also with a

range of clinical features, including the age of onset, symptoms, therapeutic responsiveness, neurocognitive function and brain morphology and activity (see review by Notaras et al. 2005). About *CACNA1C* variability, the rs1006737 polymorphism has been repeatedly associated with the risk for SZ both through GWAS and meta-analysis (Trubetskoy et al. 2022; Zhu et al. 2019) and with disrupted cognitive performance (Novaes de Oliveira Roldan et al., 2023) and altered brain structure and function in subjects with SZ (Gurung and Prata 2015). However, whether epistatic effects among these genes are involved in the aetiology of SZ remains largely unexplored. Two preceding studies have suggested that *NRN1* x *BDNF*-rs6265 epistasis is associated with the susceptibility for SZ-spectrum disorders and modulates depressive symptoms in healthy subjects (HS) (Fatjó-Vilas et al. 2016; Prats et al. 2017). In summary, the currently available molecular and genetic association data point towards the potential synergistic role of *NRN1, BDNF* and *CACNA1C* in the risk of SZ and the modulation of the disorder's presentation.

Consequently, we hypothesised that genetic epistasis between *NRN1, BDNF* and *CACNA1C* would be associated with the clinical manifestations of the disorder and that such effects would be mediated by their modulation of brain cortical structure. To test this hypothesis, first, we explored the impact of polymorphic variability along the *NRN1* gene in combination with *BDNF* (rs6265) or *CACNA1C* (rs1006737) on (i) the risk for SZ; (ii) the clinical severity and functionality plus age at onset; and, (iii) the neuroanatomical cortical measures (thickness (CT), surface area (CSA) and volume (CV)). Second, we investigated whether those brain clusters under a significant epistatic effect might be mediating the clinical outcomes of the patients.

Methods

Sample

The sample consisted of a case-control dataset of 175 individuals: 86 healthy subjects (HS) and 89 subjects diagnosed with SZ. All participants provided a biological sample for genotypic analyses and underwent a comprehensive clinical evaluation and a magnetic resonance imaging (MRI) session, detailed in the subsequent sections. The subjects with SZ were recruited from Germanes Hospitalaries psychiatric hospitals in the Barcelona area (Hospital Benito Menni and Hospital Sant Rafael), and the healthy controls were recruited from the same area.

All participants were of European origin with ages between 18 and 65 years, had an Intelligence Quotient (IQ) > 75 according to the Wechsler Adult Intelligence Scale III (WAIS-III) (Wechsler 1997) and were right-handed. Experienced psychiatrists evaluated patients using the Structured Clinical Interview for DSM Disorders (SCID) (First et al. 2002) and met the DSM-IV-TR criteria for SZ. The HS had no personal history of mental disorders or treatment. All participants met the same exclusion criteria, which included a major medical illness affecting brain function, neurological conditions, a history of head trauma with loss of consciousness and a history of drug abuse or dependence.

Ethical approval was obtained from the research ethics committee, and all participants provided written consent after being informed about the study procedures and implications. All procedures were carried out according to the Declaration of Helsinki.

Clinical assessment

The patients with SZ underwent a clinical evaluation including (i) the age at onset of the first episode, based on the first appearance of psychotic symptoms and established by experienced psychiatrists through clinical information derived from case notes and information provided by the patient and close relatives; (ii) symptom severity, evaluated with the Positive and Negative Symptoms Scale (PANSS) (Kay et al. 1987); (iii) the Global Assessment of Functioning (GAF) (Endicott et al. 1976); and, (iv) the Clinical Global Impression scale (CGI) (Table 1).

Genotyping

Genomic DNA was obtained for all individuals from buccal mucosa through cotton swabs and extracted using ATP Genomic DNA Mini Kit Tissue (Teknokroma Analítica, S.A., Sant Cugat del Vallès, Barcelona, Spain) or peripheral blood cells by punction and extracted using Realpure SSS Kit for DNA Extraction (Durviz, S.L.U, Valencia, Spain). We genotyped: (i) eleven single nucleotide polymorphisms (SNP) at the Neuritin1 gene (*NRN1*, 6p25.1), (ii) the SNP rs6265 (also known as Val66Met; GRCh38 position: 27,658,369) at the Brain-Derived Neurotrophic Factor gene (*BDNF*, 11p13) for which the T allele encodes for the amino acid methionine (Met) and the C allele encodes for valine (Val), and (iii) the SNP rs1006737 (GRCh38 position: 2,236,129) at calcium voltage-gated channel subunit alpha1 gene (*CACNA1C*, 12p13.33). The genotyping was carried out at the National Genotyping Center (CeGen) of the Network Platform of Biomolecular and Bioinformatics Resources (PRB3) of the Carlos III Health Institute (ISCIII). The genotyping call rate was 97% and, as shown in Table 2, the minor allele frequencies were comparable to the ones

Table 1 Sample characteristics, including a demographic and clinical description of the healthy subjects and the subjects diagnosed with schizophrenia (SZ) of the study

	Healthy subjects ($n=86$)	Subjects with SZ ($n=89$)	
Sex (females / males)	49/37 (57)	26/63 (29)	$X^2=13.78$; $p<0.001$
Age at interview	38.57 (11.02)	39.47 (10.88)	t=-0.55; $p=0.587$
Illness duration [a]	-	16.67 (11.01)	-
CPZ equivalents [b]	-	630 (504.91)	-
ICV	1517379.54 (152541.18)	1554805.55 (146504.42)	t=-1.656; $p=0.100$
Age of onset [a]	-	22.60 (6.38)	-
PANSS Positive	-	17.34 (5.95)	-
PANSS Negative	-	20.47 (7.68)	-
PANSS General Psychopathology	-	33.56 (9.77)	-
PANSS Total	-	71.37 (19.66)	-
GAF	-	46.33 (15.13)	-
CGI	-	4.54 (1.05)	-

Mean and standard deviation (sd) are reported for the quantitative variables, while count and percentage (%) are given for the qualitative variables. For patients, illness duration is shown in years, and chlorpromazine (CPZ) equivalents are in mg/day. Regarding clinical evaluation, the age at onset in years, the subscales of the Positive and Negative Symptoms Scale (PANSS), the Global Assessment of Functioning (GAF) scale and the Clinical Global Impression (CGI) scale are reported. Concerning neuroimaging assessment, intracranial volume (ICV) is given in mm^3 for patients and controls

[a] Data of age at onset was available for 86 subjects with SZ. Illness duration was quantified as the patient's chronological age minus the age of onset

[b] Data of CPZ equivalent was available for 85 subjects with SZ

Table 2 Information on *NRN1*, *BDNF* and *CACNA1C* SNPs that were included in this study

Gene	SNPs	Allele[a]	MAF_{1000G}	MAF_{sample}	HS genotypic counts (%)[b]			SZ genotypic counts (%)[b]		
NRN1	rs2208870	G/A	0.34	0.33	7(0.08)	43(0.5)	36(0.42)	10(0.11)	42(0.47)	37(0.42)
	rs12333117	T/C	0.40	0.40	15(0.18)	34(0.4)	36(0.42)	16(0.18)	42(0.47)	31(0.35)
	rs582186	A/G	0.38	0.39	12(0.14)	41(0.48)	32(0.38)	15(0.17)	43(0.49)	30(0.34)
	rs645649	C/G	0.36	0.38	10(0.12)	47(0.55)	29(0.34)	11(0.12)	49(0.55)	29(0.33)
	rs582262	C/G	0.30	0.28	9(0.1)	35(0.41)	42(0.49)	6(0.07)	35(0.4)	46(0.53)
	rs3763180	T/G	0.46	0.46	25(0.29)	42(0.49)	18(0.21)	14(0.16)	36(0.4)	39(0.44)
	rs10484320	T/C	0.22	0.22	5(0.06)	23(0.27)	58(0.67)	9(0.1)	33(0.37)	47(0.53)
	rs4960155	C/T	0.49	0.52	26(0.33)	40(0.5)	14(0.18)	19(0.21)	40(0.45)	30(0.34)
	rs9379002	G/T	0.29	0.26	6(0.07)	28(0.33)	51(0.6)	7(0.08)	35(0.4)	46(0.52)
	rs9405890	C/T	0.31	0.33	8(0.09)	33(0.38)	45(0.52)	14(0.16)	39(0.44)	36(0.4)
	rs1475157	G/A	0.17	0.15	4(0.05)	15(0.17)	67(0.78)	3(0.03)	23(0.26)	63(0.71)
BDNF	rs6265	T/C	0.20	0.21	2(0.02)	27(0.31)	57(0.66)	5(0.06)	34(0.38)	50(0.56)
CACNA1C	rs1006737	A/G	0.32	0.29	11(0.13)	29(0.34)	46(0.53)	9(0.1)	33(0.37)	47(0.53)

The table reports on the dbSNP number, the alleles, and the minor allele frequency (MAF) for each SNP according to 1000 Genomes Project (1000G) Phase 3 in European (EUR) population. The MAF and the genotypic count (frequencies) observed in the study sample are also given for each SNP

[a] The minor allele described in the 1000 Genomes Project for the EUR population is placed first

[b] The homozygous for the minor allele according to the EUR population of the 1000 Genomes project is placed first, then the heterozygous, and last the homozygous for the major allele

described for the EUR population in the 1,000 Genomes Project, and the genotype frequencies were in Hardy-Weinberg equilibrium (PLINK v.1.07) (Purcell et al. 2007).

MRI data acquisition, processing, and analyses

The neuroimaging protocol was conducted at the Hospital Sant Joan de Déu using a 1.5T GE Sigma MRI scanner. High-resolution structural-T1 imaging was obtained using the following acquisition parameters: matrix size = 512 × 512; 180 contiguous axial slices; voxel-resolution 0.47 × 0.47 × 1 mm3; echo (TE), repetition (TR) and inversion (TI) times, (TE / TR / TI) = 3.93 / 2000 / 710 ms, respectively; flip angle of 15°. This scan was used to estimate the three cortical measures: cortical thickness (CT), cortical surface area (CSA) and cortical volume (CV).

Structural MRI data were analysed with the FreeSurfer (http://surfer.nmr.mgh.harvard.edu/). Briefly, the pre-processing included the removal of non-brain tissue, automated Talairach transformation, tessellation of the grey and white

matter boundaries and surface deformation (Fischl et al. 2004), after which individual images were normalised to a common stereotaxic space. Some deformation procedures were performed in the data analysis pipeline, including surface inflation and registration to a spherical atlas. This method uses both intensity and continuity information from the entire three-dimensional images in the segmentation and deformation procedures to produce vertex-wise representations of CT, CSA, and CV.

All subjects included in this study passed the standardised quality-control protocols from the ENIGMA consortium (http://enigma.ini.usc.edu/protocols/imaging-protocols) that have previously been applied in large-scale multi-centre studies (Hibar et al. 2018).

Statistical analyses

Epistasis models between *NRN1* and its interactors (*BDNF* and *CACNA1C)* on SZ phenotypes

Taking into account previously reported association data, and to maximise the power of the detected gene-gene interactions, all the analyses were carried out grouping the minor and the heterozygous genotypes for all the *NRN1* SNPs, for the *BDNF*-rs6265 (Met-allele carriers and Val/Val homozygotes) and *CACNA1C*-rs1006737 (A-allele carriers and GG homozygotes; Supplementary Table S1 and S2).

First, the epistatic effects on the risk for the disorder were studied using two-way interaction factors in logistic regression models (adjusted by sex). Second, we used linear regression models to test the gene-gene effects on the clinical data within subjects with SZ (adjusted by age and sex). We only considered those interactions significant after the Bonferroni correction (0.05 / 11 = 0.0045, based on the number of SNPs analysed). Statistical power calculations were performed using version 1.0.2 of the 'genpwr' R package, considering a minor allele frequency (MAF) range of 0.17 to 0.49 and a power of 0.80. In the case of epistasis analyses on the risk (case-control logistic regression), we had a detectable odds ratio ≥ 1.85. For the epistasis analyses on the clinical measures (linear regressions within patients), we had a potentially detectable regression coefficient ≥ 1.76. Third, the impact of genetic interactions on brain measurements was explored within subjects with SZ using the Qdec graphical user interface that implements the general linear models. Specifically, our epistasis analyses were based on the two-factor, two-level ANOVA implemented in the FreeSurfer software, with the two genes (*NRN1* and the interactor, *BDNF* or *CACNA1C*) as groups and three covariates (sex, age, and intracranial volume (ICV)). We used a Monte Carlo Null-Z simulation with a z-value threshold of 2.3 (equivalent to $p < 0.005$, two tails) to correct for multiple comparisons. In these analyses, according to the Bonferroni threshold mentioned before, only those clusters with cluster-wise p-threshold that met that criterion were considered. Anatomical locations of the significant regions were determined using the surface atlas included in the FreeSurfer software. Fourth, the values for each significant cluster were imported to SPSS to explore their association with clinical measures using linear regression models (adjusted by age and sex), for graphical purposes and further moderated mediation analyses.

Moderated mediation analyses

A moderated mediation model included those epistatic models significantly associated with clinical profiles and neuroanatomical measures (Fig. 1). This approach allowed us to investigate whether those brain clusters where we detected significant epistasis between *NRN1* and *BDNF* or *CACNA1C* might mediate the effect detected on clinical profiles. We built a moderated mediation analysis with *NRN1* as the predictor variable (X), clinical features as the response variable (Y), *BDNF*-rs6265 or *CACNA1C*-rs1006737 as the moderator variable (Mo), and the brain measures as the mediating variable (Me). We used the R package "mediation" (Tingley et al. 2014). This tests, first, if the effect of X on the Me differs conditional to Mo (model 1: $Me = \beta0 + \beta1X + \beta2Mo + \beta3XMo + \varepsilon$), and second, if Me and the interaction between X and Mo are significantly associated with Y (model 2: $Y = \beta0 + \beta1Me + \beta2X + \beta3Mo + \beta4XMo + \varepsilon$). Lastly, it allows the calculation of the indirect effect (the index of moderation mediation), which is quantified as the product of the regression coefficient for the X and Mo interaction in model 1 and the regression coefficient of Me in model 2 ($\beta indirect = \beta3XMo * \beta1Me$). Once the indirect effect is calculated, the confidence interval and the significance levels for the entire indirect effect are estimated based on the bootstrap method with 10,000 resampling iterations. The conceptual and statistical diagrams of the model are shown in Fig. 1.

Results

Sample characteristics

Table 1 shows the main sociodemographic and clinical data of the sample. As shown, subjects with SZ and HS presented sex differences; then, this variable was used as a covariable in the analyses exploring the epistatic effect on the risk.

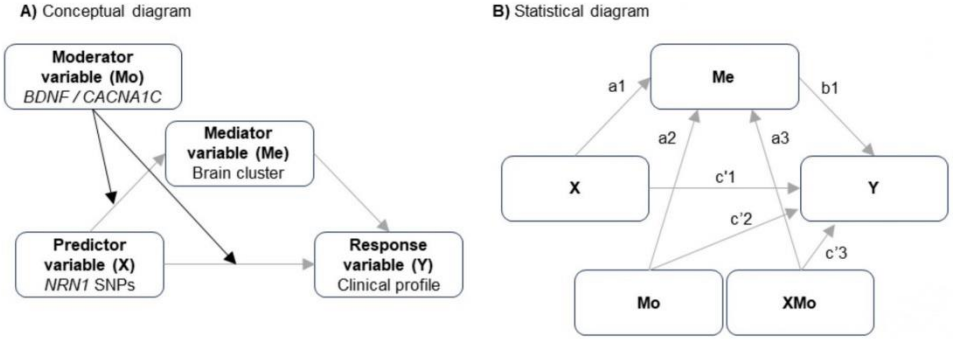


Fig. 1 Schematic representation of the different moderated mediation models used. **(A)** Conceptual diagrams representing the used model. The grey arrows indicate the indirect effect of X on Y through Me and modulated by Mo. **(B)** Statistical diagram depicting the different equations used in the moderated mediation model. Two equations are used to estimate the index of moderation mediation (IMM): first, the effect of X (a1) and Mo (a2) variables and their interaction (a3) on Me (model 1: $Me = \beta0 + \beta1X + \beta2Mo + \beta3XMo + \varepsilon$); second, the effect of X (c'1), Mo (c'2), their interaction (c'3) and Me (b1) on Y (model 2: $Y = \beta0 + \beta1Me + \beta2X + \beta3Mo + \beta4XMo + \varepsilon$). The different letters represent the coefficients of each one of these effects. Then, the index of moderated mediation was calculated as the product of the coefficients a3*b1

Table 3 Linear regression models showing significant *NRN1* epistasis with *BDNF* or *CACNA1C* on clinical measures

Clinical outcomes	Models	Genetic predictors	β	SE	p-value
Epistasis with *BDNF*					
PANSS General Psychopathology [a]	i) Main effects	*NRN1*-rs10484320	-0.087	2.105	0.426
		BDNF-rs6265	-0.018	2.146	0.873
	ii) Interaction	*NRN1*-rs10484320 *x BDNF*-rs6265	-0.583	4.076	0.003
Epistasis with *CACNA1C*					
GAF [b]	i) Main effects	*NRN1*-rs4960155	0.111	3.468	0.312
		CACNA1C-rs1006737	-0.100	3.284	0.346
	ii) Interaction	*NRN1*-rs4960155 x *CACNA1C*-rs1006737	0.233	6.727	0.002

Each factor effect is given (β, standardized regression coefficient; SE, standard error), and the global statistics of each model are shown below the table. PANSS: positive and negative syndrome scale; GAF: Global Assessment of Functioning

[a] Overall Statistical Model: i) Main effects model F = 0.605 and Adj-R^2 = 0.018, ii) Interaction model F = 2.397 and Adj-R^2 = 0.074

[b] Overall Statistical Model: i) Main effects model F = 0.414 and Adj-R^2 = 0.027, ii) Interaction model F = 2.504 and Adj-R^2 = 0.079

Epistatic effects on the risk for SZ

There were no *NRN1* x *BDNF*-rs6265 nor *NRN1* x *CACNA1C*-rs1006737 epistatic effects on the risk for the disorder (Supplementary Table S1 and S1).

Epistatic effects on SZ clinical measures

We detected a significant two-order gene-gene interaction between *NRN1*-rs10484320 and *BDNF*-rs6265 on PANSS general psychopathology. As shown in Table 3, while the main effects of *NRN1*-rs10484320 and *BDNF*-rs6265 were not detected, the interaction of both was significant. Adding the interaction term significantly improved the model's overall fit (Δ-R^2 = 0.098, p-value of the change = 0.003). As illustrated in Fig. 2A, patients presented inverse patterns in PANSS general psychopathology scores as a function of both *NRN1* and *BDNF* genotypes. Remarkably, the significance of this effect is amplified with the inclusion of treatment, as chlorpromazine (CPZ) equivalents in mg/day, as a covariate in the model (β = -0.651, SE = 4.154, p = 0.001, model Adj-R^2 = 0.099).

We also found a significant *NRN1*-rs4960155 x *CACNA1C*-rs1006737 epistasis effect on GAF scores. As indicated in Table 3, no main genotypic effects were detected, whereas their interaction was significant. When the interaction term was included, the model's overall fit significantly improved (Δ-R^2 = 0.112, p-value of the change = 0.002). As shown in Fig. 2B, patients presented inverse patterns in GAF scores as a function of both *NRN1* and *CACNA1C* genotypes. This effect did not substantially change when CPZ was added as a covariable in the model (β = 0.582, SE = 6.832, p = 0.003, model Adj-R^2 = 0.102).

Epistatic effects on SZ brain cortical measures

Regarding the interaction between *NRN1* SNPs and *BDNF*-rs6265, we detected many significant effects affecting the CSA of frontal, parietal and temporal regions. We also

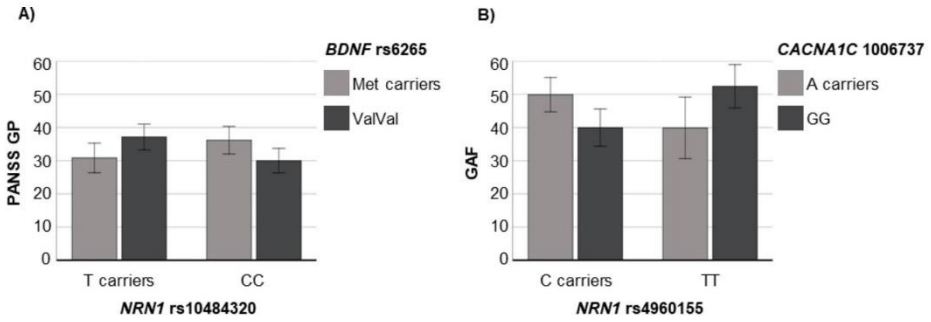


Fig. 2 Bar plots showing the significant epistatic effects detected on clinical measures within SZ subjects. Each bar represents the scores' marginal mean (±2 standard error) for each epistatic group. **A**) *NRN1*-rs10484320 x *BDNF*-rs6265 interaction on Positive and Negative Symptoms Scale (PANSS) general psychopathology. **B**) *NRN1*-rs4960155 x *CACNA1C*-rs1006737 interaction on Global Assessment of Functioning (GAF)

Table 4 Significant clusters of interaction between *NRN1* and *BDNF* on different cortical measures (CM), such as cortical surface area (CSA), cortical thickness (CT) and cortical volume (CV)

Genetic variants	CM	CN	H	MNI coordinates			Z	CWP	Size	Label
Epistasis with *BDNF*										
NRN1-rs2208870 x *BDNF*-rs6265	CSA	1	R	22.4	-94.3	12.3	3.86	0.0002	796.61	Lateral occipital
NRN1-rs4960155 x *BDNF*-rs6265	CSA	2	L	-40.3	13.8	44.8	-5.36	0.0005	813.94	Caudal middle frontal
NRN1-rs9379002 x BDNF-rs6265	CSA	3	L	-52.3	21.6	15.0	4.64	0.0025	646.83	Parsopercularis
	CSA	4	R	52.6	-11.5	37.1	5.94	0.0001	969.25	Postcentral
	CSA	5	R	16.7	38.2	-17.9	5.07	0.0001	833.06	Lateral orbitofrontal
NRN1-rs1475157 x *BDNF*-rs6265	CSA	6	L	-46.9	-40.8	-15.4	4.34	0.0001	1284.28	Inferior temporal
	CSA	7	R	7.7	47.5	37.3	4.57	0.0033	342.39	Superior frontal
NRN1-rs10484320 x *BDNF*-rs6265	CV	8	L	-27.3	21.7	-4.1	-6.33	0.0013	529.04	Lateral orbitofrontal
NRN1-rs4960155 x *BDNF*-rs6265	CV	9	L	-40.3	13.0	40.1	-5.05	0.0001	756.48	Caudal middle frontal
NRN1-rs9379002 x *BDNF*-rs6265	CV	10	R	20.5	30.4	-13.6	4.56	0.0019	499.85	Lateral orbitofrontal
Epistasis with *CACNA1C*										
NRN1-rs1475157 x *CACNA1C*-rs1006737	CSA	11	L	-47.7	-12.0	-29.4	4.48	0.0025	771.82	Inferior temporal
NRN1-rs12333117 x *CACNA1C*-rs1006737	CV	12	L	-44.2	26.9	25.6	-3.67	0.0005	576.61	Rostral middle frontal

For each cluster are given per hemisphere (H: left (L) and right (R)), with the cluster number (CN), the main peak MNI coordinates, the Z value, the cluster-wise p-value (CWP), the size (as the number of vertices), and the label according to the Deskian-Killiany atlas included in FreeSurfer

found that some epistasis effects regulated the CV of frontal regions (Table 4; Fig. 3).

Concerning the interaction between *NRN1* SNPs and *CACNA1C*-rs1006737, we detected significant effects modulating the CSA of temporal regions and the CV of frontal regions (Table 4; Fig. 4).

All the results remained significant after incorporating CPZ as a covariate in the model (data not shown).

Cortical measures effects on clinical features

We found a significant association between the cluster comprising the left lateral orbitofrontal cortex (L-LOFC) (cluster 8) and PANSS subscales and total scores (Table 5). As illustrated in Fig. 5A, B and C, greater volumes of L-LOFC were associated with elevated PANSS scores, which are indicative of more severe symptoms. These effects ramined significant when CPZ was incorporated as a covariate in the model (Positive $\beta = 0.322$, SE = 0.003, $p = 0.004$, model Adj-$R^2 = 0.063$; Negative $\beta = 0.269$, SE = 0.004, $p = 0.014$, model Adj-$R^2 = 0.105$; General Psychopathology $\beta = 0.281$, SE = 0.005, $p = 0.011$, model Adj-$R^2 = 0.059$; Total scores $\beta = 0.340$, SE = 0.009, $p = 0.001$, model Adj-$R^2 = 0.116$). We also observed a significant correlation between the inferior temporal region (cluster 11) and GAF scores (Table 5). As depicted in Fig. 5D, greater volumes of the inferior temporal cortex were associated with lower GAF values, which are indicative of worse functioning. This effect did not change when CPZ was added as a covariate in the model ($\beta = -0.243$, SE = 0.006, p = 0.024, model Adj-R2 = 0.082).

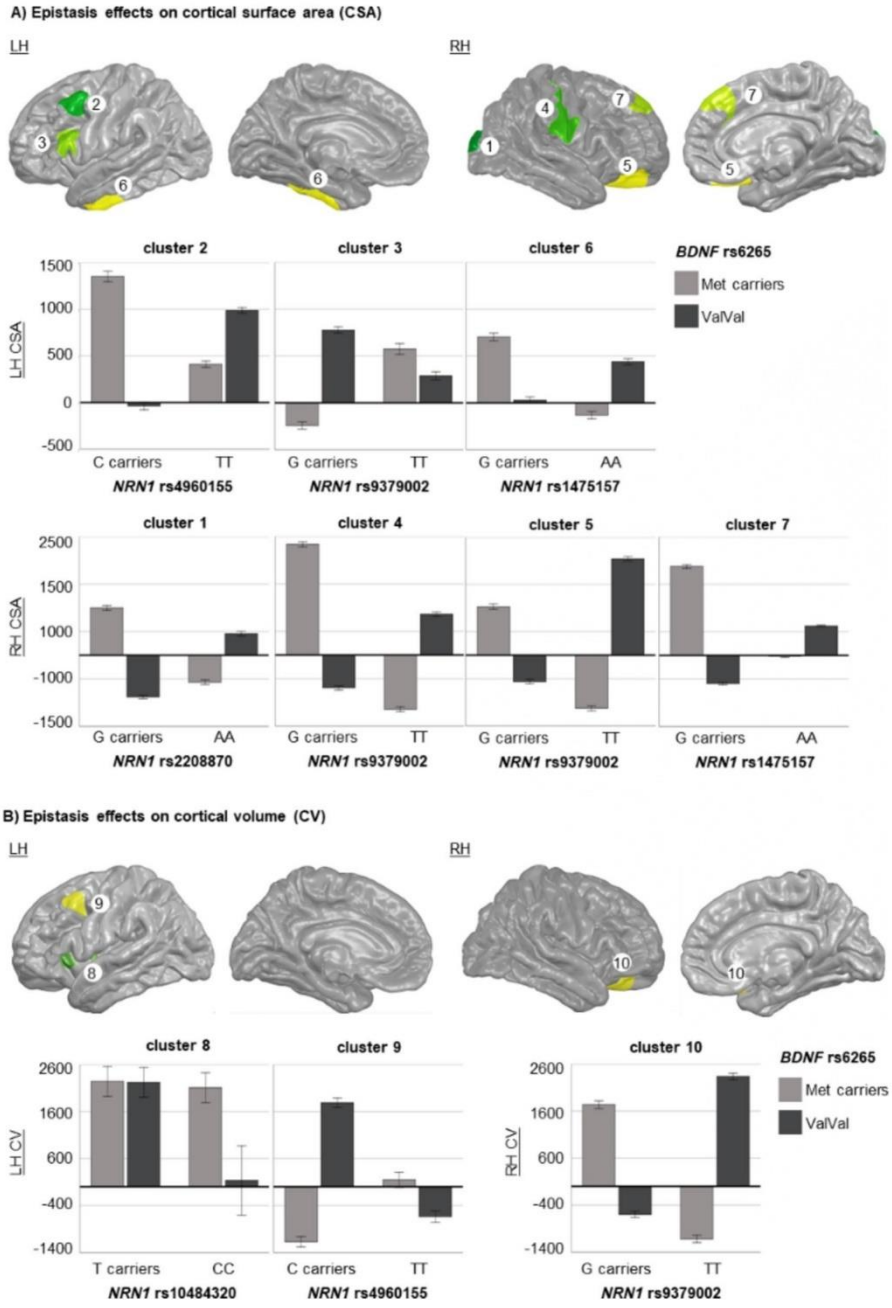


Fig. 3 Brain regions where significant epistatic effects between *NRN1* and *BDNF* were detected in the whole-brain FreeSurfer analyses. We represented significant gene-gene interactions related to cortical surface area (CSA) (**A**) and cortical volume (CV) (**B**) on the pial surface, where a specific colour distinguishes each cluster, and both lateral and medial views are presented for each hemisphere (LH: left hemisphere; RH: right hemisphere). The bar plots depict the marginal mean scores (±2 standard error) for each epistatic group

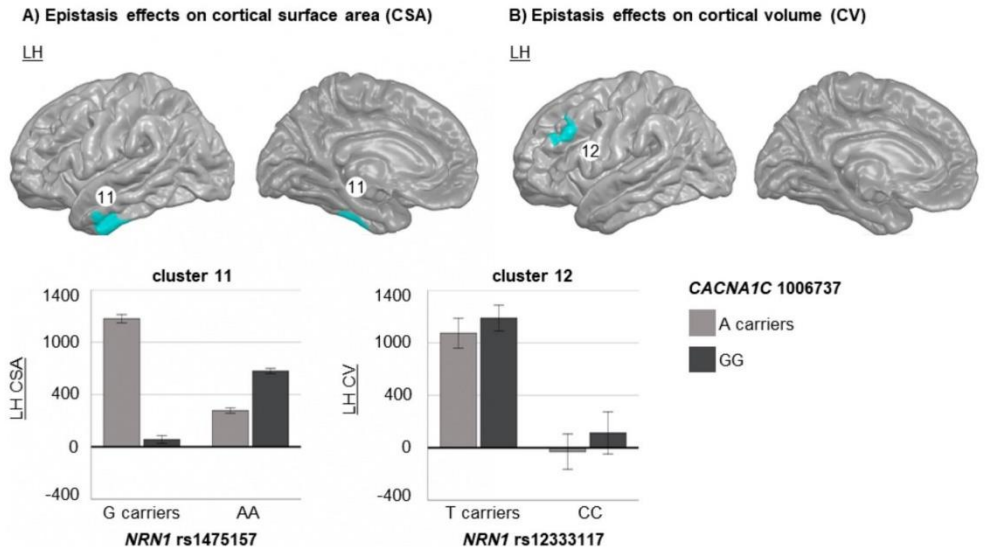


Fig. 4 Brain regions where significant epistatic effects between *NRN1* and *CACNA1C* were detected in the whole-brain FreeSurfer analyses. We represented significant gene-gene interactions related to cortical surface area (CSA) (**A**) and cortical volume (CV) (**B**) on the pial surface, where each cluster is distinguished by a specific colour, and both lateral and medial views are presented for each hemisphere (LH: left hemisphere; RH: right hemisphere). The bar plots depict the marginal mean scores (±2 standard error) for each epistatic group

Table 5 Linear regression models showing significant associations between brain clusters and clinical measures

Clinical outcomes	Cluster predictors			β	SE	Adj-R^2	p-value
	CM	CN	Label				
PANSS Positive	CV	8	Lateral orbitofrontal	0.296	0.003	0.054	0.007
PANSS Negative	CV	8	Lateral orbitofrontal	0.227	0.004	0.091	0.038
PANSS General Psychopathology	CV	8	Lateral orbitofrontal	0.250	0.005	0.050	0.021
PANSS Total	CV	8	Lateral orbitofrontal	0.303	0.010	0.085	0.005
GAF	CSA	11	Inferior temporal	-0.269	0.006	0.031	0.021

The table shows the cortical measures (CM), such as cortical volume (CV) and cortical suface area (CSA), the cluster number (CN) and its location (label) according to the Desikan-Killian Atlas; the clinical outcomes (Positive and Negative syndrome scale (PANSS) and Global Assessment Functioning (GAF)); the statistical parameters of each regression model (β, standardized regression coefficient; SE, standard error; Adj-R^2, adjusted R^2)

Brain structure as a mediator of the epistatic effects on clinical features in SZ

Given the *NRN1*-rs10484320 x *BDNF*-rs6265 epistatic effect on both PANSS general psychopathology (Table 3; Fig. 2A) and the CV of the L-LOFC (Table 4; Fig. 3, cluster 8), we explored whether this brain cluster mediated the detected epistatic effect on psychopathology. As shown in Table 6 and schematised in Fig. 6, after extracting the mean values of the L-LOFC cluster for each individual, we confirmed that *NRN1*-rs10484320 x *BDNF*-rs6265 epistasis significantly accounted for variation of the L-LOFC volume. However, when the *NRN1*-rs10484320 x *BDNF*-rs6265 interaction and the L-LOFC volume were included in the same model to predict PANSS general psychopathology, the mediator was significantly associated, but the interaction did not retain its significance. The significant index of moderated mediation indicates that the influence of *NRN1*-rs10484320 x *BDNF*-rs6265 on PANSS general psychopathology is entirely mediated by its impact on L-LOFC volume. This effect remained significant when CPZ was added as a covariate in the model (IMM = -25.839; SE = 13.942; Bootstrap 95% CI [-59.225 – -5.108], $p = 0.024$)

Discussion

Based on data coming from several animal and cellular studies that highlight the molecular links between *NRN1, BDNF* and *CACNA1C* genes (Fujino et al. 2003; Wibrand

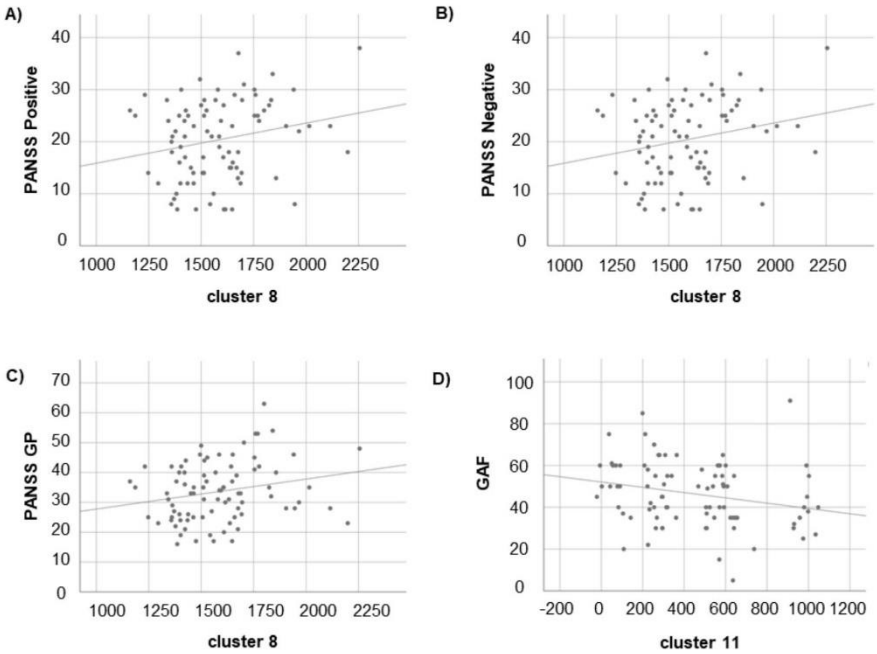


Fig. 5 Scatter plots showing the significant correlations between brain clusters and clinical measures within subjects with SZ. For both clinical and morphometric measures, the reported values signify estimated marginal means. These means have been adjusted for potential confounding factors, including age and sex. In the case of brain measures, the adjustments also encompass intracranial volume. PANSS: positive and negative syndrome scale; GP: general psychopathology; GAF: Global Assessment Functioning; CV: cortical volume; CSA: cortical surface area; L-LOFC: left lateral orbitofrontal cortex

Table 6 Moderated mediation effect of left lateral orbitofrontal cortex (L-LOFC, cluster 8) volume on the impact of *NRN1*-rs10484320 x *BDNF*-rs6265 epistasis on the Positive and Negative Symptoms Scale General Psychopathology (PANSS-GP)

Outcome variable	Models	Predictor variables	β / IMM*	SE	Bootstrap 95% CI	p-value
Mediator						
L-LOFC	a1	*NRN1*-rs10484320	-0.308	86.681	-295.80 – 21.14	0.124
	a2	*BDNF*-rs6265	-0.048	84.922	-175.477 – 118.905	0.812
	a3	*NRN1*-rs10484320 *x BDNF*-rs6265	-4.117	477.362	-2912.76 – -1070.41	0.002
Response variable						
PANSS PG	c'1	*NRN1*-rs10484320	0.367	2.980	1.429 – 12.595	0.026
	c'2	*BDNF*-rs6265	0.294	2.994	0.494 – 11.202	0.046
	c'3	*NRN1*-rs10484320 *x BDNF*-rs6265	-0.911	24.842	-68.574 – 51.022	0.401
	b1	L-LOFC	0.273	0.005	0.002 – 0.025	0.016
Moderated mediation						
NRN1-rs10484320 *x BDNF*-rs6265 → L-LOFC → PANSS PG			-24.24*	13.905	-59.132 – -3.467	0.036

The table shows the statistical parameters of each model (β, standardized regression coefficient; SE, standard error; 95% confidence interval (CI); IMM, index of moderated mediation)

et al. 2006), this study explores the neurobiological pathways through which the epistasis between these synaptic plasticity-related genes may be associated with the clinical presentation of SZ. By combining genetic, neuroimaging and clinical data, the main findings show the epistasis between *NRN1* and its interactors (*BDNF* and *CACNA1C)* on SZ clinical features and indicate that this joint genetic background may exert its effect through the impact on neuroanatomical measures.

Regarding our analyses exploring the epistatic effect on the risk for SZ, we did not detect any significant association. While the individual effect of *NRN1*, *BDNF* and *CACNA1C* on the risk for SZ is reported in different studies, there is little evidence showing that *NRN1* role seems to be not

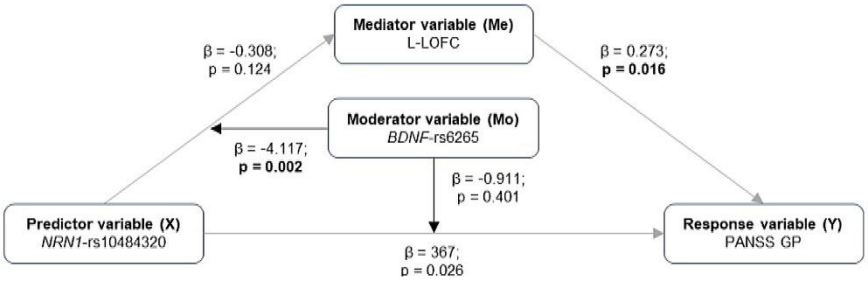


Fig. 6 Conceptual diagram showing the significant moderated mediation model. Epistatic effects between *NRN1*-rs10484320 x *BDNF*-rs6265 on the left lateral orbitofrontal cortex (L-LOFC) volume mediates the epistatic effects on Positive and Negative Symptoms Scale (PANSS) General Psychopathology

independent of *BDNF* (Fatjó-Vilas et al. 2016; Prats et al. 2017), and no previous study has explored the epistasis with *CACNA1C*. Thus, the counterpart to the novelty in our study is the challenge in comparability, as there is only one prior study (Fatjó-Vilas et al. 2016) that described a significant interaction effect between *NRN1*-rs9379002 and *BDNF*-rs6265 on a broader clinical spectrum, including both SZ and bipolar disorder. These discrepancies could be due to differences in diagnosis and sample size. Nonetheless, the current results do not indicate a direct epistatic effect on the risk of SZ, but reveal significant epistatic effects relevant to the clinical presentation of the disorder.

In this sense, we found a significant interaction between *NRN1*-rs10484320 and *BDNF*-rs6265 impacting PANSS general psychopathology, which encompasses symptoms like anxiety, guilt, tension, depression, and disorientation (Kay et al. 1987). Albeit earlier studies noted the negative impact of *BDNF*-rs6265 ValVal genotype on various PANSS subscales (Chang et al. 2009; Numata et al. 2006; Zhai et al. 2013), our data revealed that *NRN1*-rs10484320 modifies this effect. We observed more severe symptoms in patients carrying the *NRN1* T allele and the *BDNF* ValVal genotype or the *NRN1* CC genotype and the *BDNF* Met allele than in those carrying the opposite genotypic combinations. We also identified a significant interaction between *NRN1*-rs4960155 and *CACNA1C*-rs1006737 affecting GAF scores, summarising personal, social, and psychological functioning (Endicott et al. 1976). Again, previous research has established the *CACNA1C*-rs1006737 A allele as a risk factor for SZ through GWAS and meta-analytic approaches (Liu et al. 2020; Trubetskoy et al. 2022) and has demonstrated its detrimental effect on longitudinal GAF scores and recovery after psychotic episodes (Heilbronner et al. 2015). Our data adds to such evidence by revealing that *NRN1*-rs4960155 modulates the *CACNA1C* effects. Patients with the *NRN1* TT genotype and the A allele for *CACNA1C* and those with the *NRN1* GG genotype but carrying the C allele for *CACNA1C* exhibited poorer functioning. It is worth mentioning that both *NRN1* SNPs, rs10484320 and rs4960155, have been previously associated with the risk of SZ-spectrum disorders and have been found to influence IQ scores among these individuals (Chandler et al. 2010; Fatjó-Vilas et al. 2016).

Nevertheless, taking into account the clinical heterogeneity of SZ and its complex aetiology, expecting a direct influence of genetic background on the disorder's clinical presentation seems unrealistic; instead, genetic networks might modulate a lower-level trait, which, in turn, sustains the manifestation of symptoms (Glahn et al. 2010; Meijer et al. 2021). Therefore, following other previous studies (Kirschner et al. 2020; Miranda et al. 2019; Sudre et al. 2020), we have proposed that deconstructing SZ into biologically validated and stable trait markers, such as brain structural measures and investigating their role in mediating symptomatology could help to fill the gap in the path from synaptic plasticity genetic variability to the complex and heterogeneous clinical presentation of the disorder.

In this regard, we discovered that *NRN1*-rs2208870 and several upstream variants (rs10484320, rs4960155, rs9379002, rs1475157) interact with *BDNF*-rs6265 to modulate CSA and CV among individuals with SZ. Additionally, we identified that *NRN1*-rs1475157 and *NRN1*-12333117 interact with *CACNA1C*-rs1006737, modulating the CSA and CV, respectively, among individuals with SZ. These findings highlight the role of epistatic interactions involving *NRN1*, *BDNF*, and *CACNA1C* in contributing to the strong genetic underpinnings of CSA, which is estimated to have a heritability of 91% (Eyler et al. 2012). The critical roles of these genes in neural development (Sasi et al. 2017; Yao et al. 2018) also support its inclusion in the pool of genetic factors influencing CSA, which is driven by regulatory elements active during prenatal cortical development (Grasby et al. 2020).

Those brain regions significantly modulated by epistasis effects, mainly frontal regions as well as some parietal and

temporal regions, have been previously reported to present CSA and CV reductions in patients with SZ (Erp et al. 2018; Madre et al. 2020; Rimol et al. 2012). Voxel-based morphometry meta-analyses have also evidenced CV reductions, especially in frontotemporal regions (Bora et al. 2011; Honea et al. 2005). Though our analysis was confined to patients with SZ patients, our findings indicate that these specific epistatic combinations distinctly impact brain structure in this group. Thus, it is plausible that these genetic interactions might also play a role in the molecular mechanisms contributing to the previously mentioned differences in cortical structure between patients and HS.

From all the epistasis effects on brain structure, we highlight the *NRN1*-rs10484320 x *BDNF*-rs6265 interaction effect on the L-LOFC volume since these genetic variants also jointly modulate PANSS general psychopathology. Our moderated mediation model was designed to deep into the relationship between these significant associations and confirmed the mediation effect of the L-LOFC in the relationship between the *NRN1*-rs10484320 x *BDNF*-rs6265 epistasis and the PANSS general psychopathology scores.

However, contrary to the expected, the same patients who presented the lowest PANSS general psychopathology scores, those carriers of the CC genotype for *NRN1*-rs10484320 and the ValVal genotype for *BDNF*-rs6265, were the ones with the smaller left L-LOFC volume. The L-LOFC, as part of a functional network including the medial prefrontal cortex (Öngür and Price 2000), plays multiple roles, such as the integration of multiple sensory information, modulation of visceral reactions, and participation in learning, prediction, and decision-making for emotional and reward-related behaviours (Kringelbach and Rolls 2004; Rolls 2004). Findings in SZ regarding structural alterations of the OFC are controversial. While some studies have reported volume reductions (Madre et al. 2020; Rimol et al. 2012), others have described increased volume of the left OFC (Lacerda et al. 2007). These incongruities are not clarified by exploring the relationship with symptoms, as both volume increase and decrease have been associated with SZ symptomatology (Baaré et al. 1999; Gur et al. 2000; Koutsouleris et al. 2008; Lacerda et al. 2007; Nakamura et al. 2008). Interestingly, proteomic studies have linked N-methyl-D-aspartate (NMDA) receptors hypofunction and disruption of calcium homeostasis with OFC volumetric alterations in SZ (Nascimento and Martins-de-Souza 2015; Velásquez et al. 2019). At the clinical level, decreasing glutamatergic neurotransmission through NMDA receptor antagonists generates and worsens psychotic traits, suggesting its significance as a major pathway for symptom development in SZ (Lewis and González-Burgos, 2007). In fact, *NRN1* expression in the cortex is regulated by Ca^{2+} signalling via the NMDA receptor (Fujino et al. 2003), and NMDA receptor-mediated neurotransmission and plasticity are particularly affected by *BDNF*-rs6265 genotype within the hippocampus and infralimbic medial prefrontal cortex (Ninan et al. 2010; Pattwell et al. 2012). A significant pathway in the development of positive and negative symptoms may result from the reduced activity of glutamate, mainly mediated through the NMDA receptor (Lewis and González-Burgos, 2007). Therefore, both neurotrophic factors might play a role in the molecular pathways underlying the volumetric differences of the L-LOFC in SZ subjects that are behaviourally reflected in general psychopathology. Thus, our findings offer insight into the previously debated results, providing evidence that these genetic factors may contribute to volumetric variability among individuals, which, in turn, may underlie the emergence of general psychopathology during lifespan.

To understand how those genetic variants modulate psychopathology through their impact on brain structure, the functional consequences of those polymorphisms must be considered. On the one hand, animal and cell-based models have demonstrated that the *BDNF*-rs6265 Met variant affects both its intracellular distribution and activity-dependent secretion (Chen et al. 2005, 2006; Chiaruttini et al. 2009; Egan et al. 2003). On the other hand, data from the GTEx Project identifies *BDNF*-rs6265 and *NRN1*-rs10484320 variants as expression quantitative trait loci (eQTLs) affecting the expression of both genes in the brain context but does not indicate an epistatic effect. However, it is essential to note that statistical interaction between polymorphisms does not necessarily imply a direct impact on gene expression; instead, the identified epistasis could reflect the influence of these polymorphisms on intermediate pathways.

Finally, we acknowledge several limitations in our study, with the primary concern being the sample size. To mitigate potential overfitting, we calculated the smallest detectable effect in our sample for both risk assessment and clinical associations and applied the Bonferroni threshold to select significant epistasis. Concerning power estimation related to brain structure analyses it is discouraged due to the inherent properties of whole-brain approaches. Additionally, power calculations using vertices inside a significant cluster may create circularity because these vertices have already been selected for having highly different values (Vul et al. 2009). Despite these challenges, we employed various methodological strategies to avoid type I errors. Firstly, we selected polymorphic variants with known associations with SZ and described functional interactions. Secondly, we applied the cluster-wise correction method to correct for multiple comparisons and selected only those clusters that met the Bonferroni criterion. Simultaneously, to enhance the robustness of our findings and avoid potential pitfalls, we connected epistatic effects on symptomatology and brain structure

through a mediation exploration. These approaches allowed for the concurrent detection of the interaction effect between *NRN1*-rs10484320 and *BDNF*-rs6265 on SZ PANSS general psychopathology and L-LOFC volume while describing that this effect on the clinical manifestation is fully mediated by brain structure. However, we must acknowledge the potential for type II errors that may have impeded the detection of epistatic effects in other regions. Another limitation is that our study did not directly investigate the molecular mechanisms underlying the genetic interactions that contribute to the clinical presentation of SZ. It is important to note that our analyses focus on identifying statistical relationships, which do not necessarily imply direct biological connections. However, the biological plausibility of our findings is supported by prior cellular and animal models describing functional interactions related to synaptic plasticity between the studied genes. Nevertheless, further studies are necessary to validate the statistical and biological impact of genetic interactions on brain structure and symptoms. Lastly, while the homogeneity of our sample concerning ethnicity and demographic variables minimises potential errors, it also restricts the generalizability of our results. Upcoming research should strive for larger samples with more diverse representations to enhance the external validity of our findings.

Conclusions

In conclusion, our study adds clinical significance to the well-described molecular relationship between *NRN1* and its molecular interactors, *BDNF* and *CACNA1C*, since we provide the first evidence of their epistatic impact on PANSS general psychopathology through its effect on L-LOFC volume specifically within subjects with SZ.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00429-024-02793-5.

Acknowledgements We are grateful to all the participants, whose generosity made possible this study. This study received funding provided by: i) Fundación Alicia Koplowitz; ii) Acadèmia de les Ciències Mèdiques i de la Salut de Catalunya i de Balears (predoctoral contract to C A-P); iii) Instituto de Salud Carlos III through the project PI18/01535 and a Miguel Servet contract to M F-V (CP20/00072), co-funded by European Regional Development Fund (ERDF)/European Social Fund "Investing in your future"; iv) Comissionat per a Universitats i Recerca del DIUE of the Generalitat de Catalunya (Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR), 2021SGR1475). The funding sources played no role in the design of the study, the collection, analysis, or interpretation of data, or the decision to submit this manuscript for publication.

Author contributions Conceptualisation: CA-P and MF-V. Funding acquisition: MM, JS-V, CG, EP-C and MF-V. Supervision: MF-V. Resources: CA-P, MG-R, MG-L, MO-I, MM and JS-V, EP-C and MF-V. Methodology: CA-P, MG-R, EP-C and MF-V. Data curation: CA-P, MG-R, EJC-R. Formal analysis and investigation: CA-P, MG-R, MG-L, LFC, BA, CG, EJC-R and MF-V. Visualization: CA-P. Writing (original draft): CA-P, MG-R and MF-V. Writing (review and editing): all authors.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

References

Almodóvar-Payá C, Guardiola-Ripoll M, Giralt-López M, Gallego C, Salgado-Pineda P, Miret S, Salvador R, Muñoz MJ, Lázaro L, Guerrero-Pedraza A, Parellada M, Carrión MI, Cuesta MJ, Maristany T, Sarró S, Fañanás L, Callado LF, Arias B, Pomarol-Clotet E, Fatjó-Vilas M (2022) NRN1 gene as a potential marker of early-onset Schizophrenia: evidence from genetic and neuroimaging approaches. Int J Mol Sci 23(13) Article 13. https://doi.org/10.3390/ijms23137456

Baaré WFC, Pol HEH, Hijman R, Mali WPT, Viergever MA, Kahn RS (1999) Volumetric analysis of frontal lobe regions in schizophrenia: relation to cognitive function and symptomatology. Biol Psychiatry 45(12):1597–1605. https://doi.org/10.1016/S0006-3223(98)00266-2

Bora E, Fornito A, Radua J, Walterfang M, Seal M, Wood SJ, Yücel M, Velakoulis D, Pantelis C (2011) Neuroanatomical abnormalities in schizophrenia: a multimodal voxelwise meta-analysis and meta-regression analysis. Schizophr Res 127(1):46–57. https://doi.org/10.1016/j.schres.2010.12.020

Callicott JH, Feighery EL, Mattay VS, White MG, Chen Q, Baranger DAA, Berman KF, Lu B, Song H, Ming G, Weinberger DR (2013) DISC1 and SLC12A2 interaction affects human hippocampal function and connectivity. J Clin Investig 123(7):2961–2964. https://doi.org/10.1172/JCI67510

Chandler D, Dragović M, Cooper M, Badcock JC, Mullin BH, Faulkner D, Wilson SG, Hallmayer J, Howell S, Rock D, Palmer LJ, Kalaydjieva L, Jablensky A (2010) Impact of Neuritin 1 (NRN1) polymorphisms on fluid intelligence in schizophrenia. Am J Med Genet Part B: Neuropsychiatric Genet 153B(2):428–437. https://doi.org/10.1002/ajmg.b.30996

Chang H-A, Lu R-B, Shy M-J, Chang C-C, Lee M-S, Huang S-Y (2009) Brain-derived neurotrophic factor Val66Met Polymorphism: Association with psychopathological symptoms of Schizophrenia? J Neuropsychiatry Clin Neurosci 21(1):30–37. https://doi.org/10.1176/jnp.2009.21.1.30

Charlson FJ, Ferrari AJ, Santomauro DF, Diminic S, Stockings E, Scott JG, McGrath JJ, Whiteford HA (2018) Global Epidemiology and burden of Schizophrenia: findings from the global burden of Disease Study 2016. Schizophr Bull 44(6):1195–1203. https://doi.org/10.1093/schbul/sby058

Chen Z-Y, Ieraci A, Teng H, Dall H, Meng C-X, Herrera DG, Nykjaer A, Hempstead BL, Lee FS (2005) Sortilin Controls Intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. J Neurosci 25(26):6156–6166. https://doi.org/10.1523/JNEUROSCI.1017-05.2005

Chen Z-Y, Jing D, Bath KG, Ieraci A, Khan T, Siao C-J, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314(5796):140–143. https://doi.org/10.1126/science.1129663

Chiaruttini C, Vicario A, Li Z, Baj G, Braiuca P, Wu Y, Lee FS, Gardossi L, Baraban JM, Tongiorgi E (2009) Dendritic trafficking of BDNF mRNA is mediated by translin and blocked by the G196A (Val66Met) mutation. Proc Natl Acad Sci USA 106(38):16481–16486. https://doi.org/10.1073/pnas.0902833106

de Novaes AC, Fernandes Júnior LCC, de Oliveira CEC, Nunes SOV (2023) Impact of ZNF804A rs1344706 or CACNA1C rs1006737 polymorphisms on cognition in patients with severe mental disorders: a systematic review and meta-analysis. World J Biol Psychiatry 24(3):195–208. https://doi.org/10.1080/15622975.2022.2097308

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112(2):257–269. https://doi.org/10.1016/S0092-8674(03)00035-7

Endicott J, Spitzer RL, Fleiss JL, Cohen J (1976) The Global Assessment Scale: A Procedure for Measuring overall severity of Psychiatric Disturbance. Arch Gen Psychiatry 33(6):766–771. https://doi.org/10.1001/archpsyc.1976.01770060086012

Esvald E-E, Tuvikene J, Kiir CS, Avarlaid A, Tamberg L, Sirp A, Shubina A, Cabrera-Cabrera F, Pihlak A, Koppel I, Palm K, Timmusk T (2023) Revisiting the expression of BDNF and its receptors in mammalian development. Front Mol Neurosci 16. https://www.frontiersin.org/articles/https://doi.org/10.3389/fnmol.2023.1182499

Eyler LT, Chen C-H, Panizzon MS, Fennema-Notestine C, Neale MC, Jak A, Jernigan TL, Fischl B, Franz CE, Lyons MJ, Grant M, Prom-Wormley E, Seidman LJ, Tsuang MT, Fiecas MJA, Dale AM, Kremen WS (2012) A comparison of heritability maps of cortical surface area and thickness and the influence of Adjustment for whole brain measures: a magnetic resonance imaging twin study. Twin Res Hum Genetics: Official J Int Soc Twin Stud 15(3):304–314. https://doi.org/10.1017/thg.2012.3

Fatjó-Vilas M, Prats C, Pomarol-Clotet E, Lázaro L, Moreno C, González-Ortega I, Lera-Miguel S, Miret S, Muñoz MJ, Ibáñez I, Campanera S, Giralt-López M, Cuesta MJ, Peralta V, Ortet G, Parellada M, González-Pinto A, McKenna PJ, Fañanás L (2016) Involvement of NRN1 gene in schizophrenia-spectrum and bipolar disorders and its impact on age at onset and cognitive functioning. World J Biol Psychiatry 17(2):129–139. https://doi.org/10.3109/15622975.2015.1093658

Finkbeiner S, Tavazoie SF, Maloratsky A, Jacobs KM, Harris KM, Greenberg ME (1997) CREB: a major mediator of neuronal neurotrophin responses. Neuron 19(5):1031–1047. https://doi.org/10.1016/S0896-6273(00)80395-5

First M, Spitzer R, Gibbon M, Williams J (2002) Structured clinical interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition. En (SCID-I/P)

Fischl B, van der Kouwe A, Destrieux C, Halgren E, Ségonne F, Salat DH, Busa E, Seidman LJ, Goldstein J, Kennedy D, Caviness V, Makris N, Rosen B, Dale AM (2004) Automatically parcellating the human cerebral cortex. Cereb Cortex 14(1):11–22. https://doi.org/10.1093/cercor/bhg087

Fujino T, Lee W-CA, Nedivi E (2003) Regulation of cpg15 by signaling pathways that mediate synaptic plasticity. Mol Cell Neurosci 24(3):538–554. https://doi.org/10.1016/S1044-7431(03)00230-6

Glahn DC, Winkler AM, Kochunov P, Almasy L, Duggirala R, Carless MA, Curran JC, Olvera RL, Laird AR, Smith SM, Beckmann CF, Fox PT, Blangero J (2010) Genetic control over the resting brain. Proceedings of the National Academy of Sciences, 107(3), 1223–1228. https://doi.org/10.1073/pnas.0909969107

Grasby KL, Jahanshad N, Painter JN, Colodro-Conde L, Bralten J, Hibar DP, Lind PA, Pizzagalli F, Ching CRK, McMahon MAB, Shatokhina N, Zsembik LCP, Thomopoulos SI, Zhu AH, Strike LT, Agartz I, Alhusaini S, Almeida MAA, Alnæs D, ENHANCING NEUROIMAGING GENETICS THROUGH META-ANALYSIS CONSORTIUM (ENIGMA)—GENETICS WORKING GROUP (2020) The genetic architecture of the human cerebral cortex. Science 367(6484):eaay6690. https://doi.org/10.1126/science.aay6690

Guardiola-Ripoll M, Almodóvar-Payá C, Lubeiro A, Sotero A, Salvador R, Fuentes-Claramonte P, Salgado-Pineda P, Papiol S, Ortiz-Gil J, Gomar JJ, Guerrero-Pedraza A, Sarró S, Maristany T, Molina V, Pomarol-Clotet E, Fatjó-Vilas M (2022) A functional neuroimaging association study on the interplay between two schizophrenia genome-wide associated genes (CACNA1C and ZNF804A). Eur Arch Psychiatry Clin NeuroSci 272(7):1229–1239. https://doi.org/10.1007/s00406-022-01447-z

Gur RE, Cowell PE, Latshaw A, Turetsky BI, Grossman RI, Arnold SE, Bilker WB, Gur RC (2000) Reduced dorsal and Orbital Prefrontal Gray Matter Volumes in Schizophrenia. Arch Gen Psychiatry 57(8):761–768. https://doi.org/10.1001/archpsyc.57.8.761

Gurung R, Prata DP (2015) What is the impact of genome-wide supported risk variants for schizophrenia and bipolar disorder on brain structure and function? A systematic review. Psychol Med 45(12):2461–2480. https://doi.org/10.1017/S0033291715000537

Hall J, Bray NJ (2022) Schizophrenia Genomics: convergence on synaptic development, adult synaptic plasticity, or both? Biol Psychiatry 91(8):709–717. https://doi.org/10.1016/j.biopsych.2021.10.018

Harwell C, Burbach B, Svoboda K, Nedivi E (2005) Regulation of cpg15 expression during single whisker experience in the Barrel cortex of adult mice. J Neurobiol 65(1):85–96. https://doi.org/10.1002/neu.20176

Heilbronner U, Malzahn D, Strohmaier J, Maier S, Frank J, Treutlein J, Mühleisen TW, Forstner AJ, Witt SH, Cichon S, Falkai P, Nöthen MM, Rietschel M, Schulze TG (2015) A common risk variant in CACNA1C supports a sex-dependent effect on longitudinal functioning and functional recovery from episodes of schizophrenia-spectrum but not bipolar disorder. Eur Neuropsychopharmacol 25(12):2262–2270. https://doi.org/10.1016/j.euroneuro.2015.09.012

Hibar DP, Westlye LT, Doan NT, Jahanshad N, Cheung JW, Ching CRK, Versace A, Bilderbeck AC, Uhlmann A, Mwangi B, Krämer B, Overs B, Hartberg CB, Abé C, Dima D, Grotegerd D, Sprooten E, Bøen E, Jimenez E, Andreassen OA (2018) Cortical abnormalities in bipolar disorder: an MRI analysis of 6503 individuals from the ENIGMA bipolar disorder Working Group. Mol Psychiatry 23(4) Article 4. https://doi.org/10.1038/mp.2017.73

Hilker R, Helenius D, Fagerlund B, Skytthe A, Christensen K, Werge TM, Nordentoft M, Glenthøj B (2018) Heritability of Schizophrenia and Schizophrenia Spectrum based on the nationwide Danish twin Register. Biol Psychiatry 83(6):492–498. https://doi.org/10.1016/j.biopsych.2017.08.017

Honea R, Crow TJ, Passingham D, Mackay CE (2005) Regional deficits in Brain volume in Schizophrenia: a Meta-analysis of Voxel-based Morphometry studies. Am J Psychiatry 162(12):2233–2245. https://doi.org/10.1176/appi.ajp.162.12.2233

Jenkins MA, Christel CJ, Jiao Y, Abiria S, Kim KY, Usachev YM, Obermair GJ, Colbran RJ, Lee A (2010) Ca2+-Dependent facilitation of Cav1.3 Ca2 + channels by Densin and Ca2+/Calmodulin-Dependent protein kinase II. J Neurosci 30(15):5125–5135. https://doi.org/10.1523/JNEUROSCI.4367-09.2010

Ji Y, Zhang X, Wang Z, Qin W, Liu H, Xue K, Tang J, Xu Q, Zhu D, Liu F, Yu C (2021) Genes associated with gray matter volume alterations in schizophrenia. NeuroImage 225:117526. https://doi.org/10.1016/j.neuroimage.2020.117526

Kaldun JC, Sprecher SG (2019) Initiated by CREB: Resolving Gene Regulatory Programs in Learning and Memory. BioEssays 41(8):1900045. https://doi.org/10.1002/bies.201900045

Kay SR, Fiszbein A, Opler LA (1987) The positive and negative syndrome scale (PANSS) for Schizophrenia. Schizophr Bull 13(2):261–276. https://doi.org/10.1093/schbul/13.2.261

Kheirollahi M, Kazemi E, Ashouri S (2016) Brain-derived neurotrophic factor gene Val66Met polymorphism and risk of Schizophrenia: a Meta-analysis of case–control studies. Cell Mol Neurobiol 36(1):1–10. https://doi.org/10.1007/s10571-015-0229-z

Kirschner M, Shafiei G, Markello RD, Makowski C, Talpalaru A, Hodzic-Santor B, Devenyi GA, Paquola C, Bernhardt BC, Lepage M, Chakravarty MM, Dagher A, Mišić B (2020) Latent clinical-anatomical dimensions of Schizophrenia. Schizophr Bull 46(6):1426–1438. https://doi.org/10.1093/schbul/sbaa097

Koutsouleris N, Gaser C, Jäger M, Bottlender R, Frodl T, Holzinger S, Schmitt GJE, Zetzsche T, Burgermeister B, Scheuerecker J, Born C, Reiser M, Möller H-J, Meisenzahl EM (2008) Structural correlates of psychopathological symptom dimensions in schizophrenia: a voxel-based morphometric study. NeuroImage 39(4):1600–1612. https://doi.org/10.1016/j.neuroimage.2007.10.029

Kringelbach ML, Rolls ET (2004) The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. Prog Neurobiol 72(5):341–372. https://doi.org/10.1016/j.pneurobio.2004.03.006

Lacerda ALT, Hardan AY, Yorbik O, Vemulapalli M, Prasad KM, Keshavan MS (2007) Morphology of the orbitofrontal cortex in first-episode schizophrenia: relationship with negative symptomatology. Prog Neuropsychopharmacol Biol Psychiatry 31(2):510–516. https://doi.org/10.1016/j.pnpbp.2006.11.022

Legge SE, Cardno AG, Allardyce J, Dennison C, Hubbard L, Pardiñas AF, Richards A, Rees E, Di Florio A, Escott-Price V, Zammit S, Holmans P, Owen MJ, O'Donovan MC, Walters JTR (2021) Associations between Schizophrenia Polygenic liability, Symptom dimensions, and cognitive ability in Schizophrenia. JAMA Psychiatry 78(10):1143–1151. https://doi.org/10.1001/jamapsychiatry.2021.1961

Liu Y, Wu X, Xia X, Yao J, Wang B (2020) The genome-wide supported CACNA1C gene polymorphisms and the risk of schizophrenia: an updated meta-analysis. BMC Med Genet 21(1):159. https://doi.org/10.1186/s12881-020-01084-0

Lu J-M, Liu D-D, Li Z-Y, Ling C, Mei Y-A (2017) Neuritin enhances synaptic transmission in Medial Prefrontal Cortex in mice by increasing CaV3.3 surface expression. Cereb Cortex 27(7):3842–3855. https://doi.org/10.1093/cercor/bhx082

Mackay TF, Moore JH (2014) Why epistasis is important for tackling complex human disease genetics. Genome Med 6(6):42. https://doi.org/10.1186/gm561

Madre M, Canales-Rodríguez EJ, Fuentes-Claramonte P, Alonso-Lana S, Salgado-Pineda P, Guerrero-Pedraza A, Moro N, Bosque C, Gomar JJ, Ortíz-Gil J, Goikolea JM, Bonnin CM, Vieta E, Sarró S, Maristany T, McKenna PJ, Salvador R, Pomarol-Clotet E (2020) Structural abnormality in schizophrenia versus bipolar disorder: a whole brain cortical thickness, surface area, volume and gyrification analyses. NeuroImage: Clin 25:102131. https://doi.org/10.1016/j.nicl.2019.102131

Ma H, Groth RD, Wheeler DG, Barrett CF, Tsien RW (2011) Excitation–transcription coupling in sympathetic neurons and the molecular mechanism of its initiation. Neurosci Res 70(1):2–8. https://doi.org/10.1016/j.neures.2011.02.004

Meijer M, Keo A, van Leeuwen JMC, Dzyubachyk O, Meijer OC, Vinkers CH, Mahfouz A (2021) Molecular characterisation of the stress network in individuals at risk for schizophrenia. Neurobiol Stress 14:100307. https://doi.org/10.1016/j.ynstr.2021.100307

Miranda GG, Rodrigue KM, Kennedy KM (2019) Fronto-parietal cortical thickness mediates the effect of COMT Val-158Met polymorphism on age-associated executive function. Neurobiol Aging 73:104–114. https://doi.org/10.1016/j.neurobiolaging.2018.08.027

Naeve GS, Ramakrishnan M, Kramer R, Hevroni D, Citri Y, Theill LE (1997) Neuritin: a gene induced by neural activity and neurotrophins that promotes neuritogenesis. Proc Natl Acad Sci USA 94(6):2648–2653

Nakamura M, Nestor PG, Levitt JJ, Cohen AS, Kawashima T, Shenton ME, McCarley RW (2008) Orbitofrontal volume deficit in schizophrenia and thought disorder. Brain 131(Pt 1):180–195. https://doi.org/10.1093/brain/awm265

Nascimento JM, Martins-de-Souza D (2015) The proteome of schizophrenia. Npj Schizophrenia 1(1). https://doi.org/10.1038/npjschz.2014.3

Nedivi E, Fieldust S, Theill LE, Hevron D (1996) A set of genes expressed in response to light in the adult cerebral cortex and regulated during development. Proc Natl Acad Sci USA 93(5):2048–2053

Öngür D, Price JL (2000) The Organization of Networks within the Orbital and Medial Prefrontal cortex of rats, monkeys and humans. Cereb Cortex 10(3):206–219. https://doi.org/10.1093/cercor/10.3.206

Ninan I, Bath KG, Dagar K, Perez-Castro R, Plummer MR, Lee FS, Chao MV (2010) The BDNF Val66Met polymorphism impairs NMDA receptor-dependent synaptic plasticity in the Hippocampus. J Neurosci 30(26):8866–8870. https://doi.org/10.1523/JNEUROSCI.1405-10.2010

Notaras M, Hill R, van den Buuse M (2005) A role for the BDNF gene Val66Met polymorphism in schizophrenia? A comprehensive review. Neurosci Biobehav Rev 51:15–30. https://doi.org/10.1016/j.neubiorev.2014.12.016

Numata S, Ueno S, Iga J, Yamauchi K, Hongwei S, Ohta K, Kinouchi S, Shibuya-Tayoshi S, Tayoshi S, Aono M, Kameoka N, Sumitani S, Tomotake M, Kaneda Y, Taniguchi T, Ishimoto Y, Ohmori T (2006) Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism in schizophrenia is associated with age at onset and symptoms. Neurosci Lett 401(1):1–5. https://doi.org/10.1016/j.neulet.2006.02.054

Pardiñas AF, Holmans P, Pocklington AJ, Escott-Price V, Ripke S, Carrera N, Legge SE, Bishop S, Cameron D, Hamshere ML, Han J, Hubbard L, Lynham A, Mantripragada K, Rees E, MacCabe JH, McCarroll SA, Baune BT, Breen G, Walters JTR (2018) Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection. Nat Genet 50(3) Article 3. https://doi.org/10.1038/s41588-018-0059-2

Pattwell SS, Bath KG, Perez-Castro R, Lee FS, Chao MV, Ninan I (2012) The BDNF Val66Met polymorphism impairs synaptic transmission and plasticity in the Infralimbic Medial Prefrontal Cortex. J Neurosci 32(7):2410–2421. https://doi.org/10.1523/JNEUROSCI.5205-11.2012

Phillips PC (2008) Epistasis–the essential role of gene interactions in the structure and evolution of genetic systems. Nat Rev Genet 9(11):855–867. https://doi.org/10.1038/nrg2452

Prats C, Arias B, Ortet G, Ibáñez MI, Moya J, Pomarol-Clotet E, Fañanás L, Fatjó-Vilas M (2017) Role of neurotrophins in depressive symptoms and executive function: Association analysis of NRN1 gene and its interaction with BDNF gene in a non-clinical sample. J Affect Disord 211:92–98. https://doi.org/10.1016/j.jad.2016.11.017

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ, Sham PC (2007) PLINK: a Tool Set for whole-genome Association and Population-based linkage analyses. Am J Hum Genet 81(3):559–575

Rimol LM, Nesvåg R, Hagler DJ, Bergmann Ø, Fennema-Notestine C, Hartberg CB, Haukvik UK, Lange E, Pung CJ, Server A, Melle I, Andreassen OA, Agartz I, Dale AM (2012) Cortical volume, Surface Area, and thickness in Schizophrenia and Bipolar Disorder. Biol Psychiatry 71(6):552–560. https://doi.org/10.1016/j.biopsych.2011.11.026

Roguev A, Bandyopadhyay S, Zofall M, Zhang K, Fischer T, Collins SR, Qu H, Shales M, Park H-O, Hayles J, Hoe K-L, Kim D-U, Ideker T, Grewal SI, Weissman JS, Krogan NJ (2008) Conservation and rewiring of functional modules revealed by an epistasis map in fission yeast. Science 322(5900):405–410. https://doi.org/10.1126/science.1162609

Rolls ET (2004) The functions of the orbitofrontal cortex. Brain Cogn 55(1):11–29. https://doi.org/10.1016/S0278-2626(03)00277-X

Rosa A, Cuesta MJ, Fatjó-Vilas M, Peralta V, Zarzuela A, Fañanás L (2006) The Val66Met polymorphism of the brain-derived neurotrophic factor gene is associated with risk for psychosis: evidence from a family-based association study. Am J Med Genet Part B: Neuropsychiatric Genet 141B(2):135–138. https://doi.org/10.1002/ajmg.b.30266

Sasi M, Vignoli B, Canossa M, Blum R (2017) Neurobiology of local and intercellular BDNF signaling. Pflügers Archiv - Eur J Physiol 469(5):593–610. https://doi.org/10.1007/s00424-017-1964-4

Sengupta SM, MacDonald K, Fathalli F, Yim A, Lepage M, Iyer S, Malla A, Joober R (2017) Polygenic risk score associated with specific symptom dimensions in first-episode psychosis. Schizophr Res 184:116–121. https://doi.org/10.1016/j.schres.2016.11.039

Striessnig J, Pinggera A, Kaur G, Bock G, Tuluc P (2014) L-type Ca2 + channels in heart and brain. Wiley Interdisciplinary Reviews: Membrane Transp Signal 3(2):15–38. https://doi.org/10.1002/wmts.102

Sudre G, Frederick J, Sharp W, Ishii-Takahashi A, Mangalmurti A, Choudhury S, Shaw P (2020) Mapping associations between polygenic risks for childhood neuropsychiatric disorders, symptoms of attention deficit hyperactivity disorder, cognition, and the brain. Mol Psychiatry 25(10). https://doi.org/10.1038/s41380-019-0350-3. Article 10

Sullivan PF, Kendler KS, Neale MC (2003) Schizophrenia as a Complex Trait: evidence from a Meta-analysis of Twin studies. Arch Gen Psychiatry 60(12):1187. https://doi.org/10.1001/archpsyc.60.12.1187

Tecelão D, Mendes A, Martins D, Fu C, Chaddock CA, Picchioni MM, McDonald C, Kalidindi S, Murray R, Prata DP (2019) The effect of psychosis associated CACNA1C, and its epistasis with ZNF804A, on brain function. Genes Brain Behav 18(4):e12510. https://doi.org/10.1111/gbb.12510

Tingley D, Yamamoto T, Hirose K, Keele L, Imai K (2014) Mediation: R Package for Causal Mediation Analysis. J Stat Softw 59:1–38. https://doi.org/10.18637/jss.v059.i05

Tongiorgi E (2008) Activity-dependent expression of brain-derived neurotrophic factor in dendrites: facts and open questions. Neurosci Res 61(4):335–346. https://doi.org/10.1016/j.neures.2008.04.013

Trubetskoy V, Pardiñas AF, Qi T, Panagiotaropoulou G, Awasthi S, Bigdeli TB, Bryois J, Chen C-Y, Dennison CA, Hall LS, Lam M, Watanabe K, Frei O, Ge T, Harwood JC, Koopmans F, Magnusson S, Richards AL, Sidorenko J, O'Donovan MC (2022) Mapping genomic loci implicates genes and synaptic biology in schizophrenia. Nature 604(7906) Article 7906. https://doi.org/10.1038/s41586-022-04434-5

van der Meer D, Kaufmann T (2022) Mapping the genetic architecture of cortical morphology through neuroimaging: progress and perspectives. Translational Psychiatry 12(1). https://doi.org/10.1038/s41398-022-02193-5. Article 1

van Erp TGM, Walton E, Hibar DP, Schmaal L, Jiang W, Glahn DC, Pearlson GD, Yao N, Fukunaga M, Hashimoto R, Okada N, Yamamori H, Bustillo JR, Clark VP, Agartz I, Mueller BA, Cahn W, de Zwarte SMC, Pol HEH, Turner JA (2018) Cortical brain abnormalities in 4474 individuals with Schizophrenia and 5098 control subjects via the Enhancing Neuro Imaging Genetics through Meta Analysis (ENIGMA) Consortium. Biol Psychiatry 84(9):644–654. https://doi.org/10.1016/j.biopsych.2018.04.023

Velásquez E, Martins-de-Souza D, Velásquez I, Carneiro GRA, Schmitt A, Falkai P, Domont GB, Nogueira FCS (2019) Quantitative subcellular proteomics of the Orbitofrontal Cortex of Schizophrenia Patients. J Proteome Res 18(12):4240–4253. https://doi.org/10.1021/acs.jproteome.9b00398

Vul E, Harris C, Winkielman P, Pashler H (2009) Voodoo correlations in social neuroscience. Perspect Psychol Sci 4(3):274–290

Wechsler D (1997) Wechsler Adult Intelligence Scale (3rd ed.)

Wibrand K, Messaoudi E, Håvik B, Steenslid V, Løvlie R, Steen VM, Bramham CR (2006) Identification of genes co-upregulated with Arc during BDNF-induced long-term potentiation in adult rat dentate gyrus in vivo. Eur J Neurosci 23(6):1501–1511. https://doi.org/10.1111/j.1460-9568.2006.04687.x

Woo HJ, Yu C, Kumar K, Reifman J (2017) Large-scale interaction effects reveal missing heritability in schizophrenia, bipolar disorder and posttraumatic stress disorder. Translational Psychiatry 7(4) Article 4. https://doi.org/10.1038/tp.2017.61

Xu Q, Xiong Y, Yuan C, Liu F, Zhao F, Shen J, Qin W, Yu C (2018) ZNF804A rs1344706 interacts with COMT rs4680 to affect prefrontal volume in healthy adults. Brain Imaging Behav 12(1):13–19. https://doi.org/10.1007/s11682-016-9671-x

Yao J-J, Gao X-F, Chow C-W, Zhan X-Q, Hu C-L, Mei Y-A (2012) Neuritin activates insulin receptor pathway to Up-regulate Kv4.2-mediated transient outward K + current in rat cerebellar granule neurons *. J Biol Chem 287(49):41534–41545. https://doi.org/10.1074/jbc.M112.390260

Yao J, Zhao Q, Lu J, Mei Y (2018) Functions and the related signaling pathways of the neurotrophic factor neuritin. Acta Pharmacol Sin 39(9):1414–1420. https://doi.org/10.1038/aps.2017.197

Zhai J, Yu Q, Chen M, Gao Y, Zhang Q, Li J, Wang K, Ji F, Su Z, Li W, Li X, Qiao J (2013) Association of the brain-derived neurotrophic factor gene G196A rs6265 polymorphisms and the cognitive function and clinical symptoms of schizophrenia. Int J Clin Exp Pathol 6(8):1617–1623

Zhao Q-R, Lu J-M, Li Z-Y, Mei Y-A (2018) Neuritin promotes neurite and spine growth in rat cerebellar granule cells via L-type calcium channel-mediated calcium influx. J Neurochem 147(1):40–57. https://doi.org/10.1111/jnc.14535

Zhu D, Yin J, Liang C, Luo X, Lv D, Dai Z, Xiong S, Fu J, Li Y, Lin J, Lin Z, Wang Y, Ma G (2019) CACNA1C (rs1006737) may be

a susceptibility gene for schizophrenia: an updated meta-analysis. Brain Behav 9(6):e01292. https://doi.org/10.1002/brb3.1292

Özsoy ED, Yılmaz M, Patlar B, Emecen G, Durmaz E, Magwire MM, Zhou S, Huang W, Anholt RRH, Mackay TFC (2021) Epistasis for head morphology in Drosophila melanogaster. G3: Genes|Genomes|Genetics, 11. 10jkab285. https://doi.org/10.1093/g3journal/jkab285

Zuk O, Hechter E, Sunyaev SR, Lander ES (2012) The mystery of missing heritability: genetic interactions create phantom heritability. Proc Natl Acad Sci 109(4):1193–1198. https://doi.org/10.1073/pnas.1119675109

Authors and Affiliations

Carmen Almodóvar-Payá[1,2,3] · Maria Guardiola-Ripoll[1,15] · Maria Giralt-López[4,5] · Maitane Oscoz-Irurozqui[1,6] · Erick Jorge Canales-Rodríguez[1,3,7] · Mercè Madre[1,8] · Joan Soler-Vidal[1,3,9] · Núria Ramiro[10] · Luis F. Callado[3,11,12] · Bárbara Arias[2,3,13] · Carme Gallego[14] · Edith Pomarol-Clotet[1,3] · Mar Fatjó-Vilas[1,2,3]

✉ Mar Fatjó-Vilas
mfatjo-vilas@fidmag.org

Carmen Almodóvar-Payá
calmodovar@fidmag.org

[1] FIDMAG Germanes Hospitalàries Research Foundation, Barcelona, Spain

[2] Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

[3] CIBERSAM (Biomedical Research Network in Mental Health), Instituto de Salud Carlos III, Madrid, Spain

[4] Department of Child and Adolescent Psychiatry, Germans Trias i Pujol University Hospital (HUGTP), Barcelona, Spain

[5] Department of Psychiatry and Legal Medicine, Faculty of Medicine, Autonomous University of Barcelona (UAB), Barcelona, Spain

[6] Red de Salud Mental de Gipuzkoa, Osakidetza-Basque Health Service, Gipuzkoa, Spain

[7] Signal Processing Laboratory (LTS5), École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

[8] Mental Health, IR SANT PAU, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma Barcelona, Barcelona, Spain

[9] Hospital Benito Menni, Germanes Hospitalàries, Sant Boi de Llobregat, Barcelona, Spain

[10] Hospital San Rafael, Germanes Hospitalàries, Barcelona, Spain

[11] Department of Pharmacology, University of the Basque Country (UPV/EHU), Bizkaia, Spain

[12] BioBizkaia Health Research Institute, Bizkaia, Spain

[13] Institut de Biomedicina de la Universitat de Barcelona (IBUB), Barcelona, Spain

[14] Department of Cells and Tissues, Molecular Biology Institute of Barcelona (IBMB-CSIC), Barcelona, Spain

[15] CIBERER (Biomedical Research Network in Rare Diseases), Instituto de Salud Carlos III, Madrid, Spain

Section II: Brain *NRN1* genetic, epigenetic, and expression correlates and the modulatory effect of antipsychotic treatment

Study II

Clozapine-related brain *NRN1* expression patterns are associated with methylation and genetic variants in schizophrenia

Almodóvar-Payá C, Moreno M, Guardiola-Ripoll M, Latorre-Guardia M, Morentin B, Garcia-Ruíz B, Pomarol-Clotet E, Callado LF, Gallego C, Fatjó-Vilas M

Submitted for peer review in an indexed journal

CLOZAPINE-RELATED BRAIN *NRN1* EXPRESSION PATTERNS ARE ASSOCIATED WITH METHYLATION AND GENETIC VARIANTS IN SCHIZOPHRENIA

Carmen Almodóvar-Payá[1,2,3,#,a], Marcos Moreno[4,#], Maria Guardiola-Ripoll[1,5], Mariona Latorre-Guardia[1,2], Benito Morentin[6,7], Beatriz Garcia-Ruíz[1,2,8], Edith Pomarol-Clotet[1,2], Luis F. Callado [2,7,9], Carme Gallego[4,+], Mar Fatjó-Vilas[1,2,3+,a].

1. FIDMAG Germanes Hospitalàries Research Foundation, Barcelona, Spain.
2. CIBERSAM (Biomedical Research Network in Mental Health; Instituto de Salud Carlos III),Spain.
3. Department of Evolutionary Biology, Ecology and Environmental Sciences, University of Barcelona (UB), Barcelona, Spain.
4. Department of Cell and Tissues, Molecular Biology Institute of Barcelona (IBMB-CSIC), Barcelona, Spain.
5. CIBERER (Biomedical Research Network in Rare Diseases; Instituto de Salud Carlos III), Spain.
6. Section of Forensic Pathology, Basque Institute of Legal Medicine, Bilbao, Spain
7. BioBizkaia Health Research Institute, Bizkaia, Spain.
8. Institut d'Investigació Sanitària Pere Virgili (IISPV)-CERCA, Tarragona, Spain
9. Department of Pharmacology, University of the Basque Country (UPV/EHU), Bizkaia, Spain.

[#] Co-first authors: C Almodóvar-Payá and M Moreno

[+] Co-senior authors: C Gallego and M Fatjó-Vilas

[a] Corresponding authors: C Almodóvar-Payá (calmodovar@fidmag.org) and M Fatjó-Vilas (mfatjo-vilas@fidmag.org)

ABSTRACT

The Neuritin-1 gene (*NRN1*), involved in neurodevelopment and synaptic plasticity, is associated with schizophrenia (SZ) and related clinical, cognitive, and neuroimaging phenotypes. Additionally, it is one of the most differentially methylated genes in the prefrontal cortex (PFC) in SZ and is responsive to neurotherapeutic agents. We aimed to investigate *NRN1*'s molecular mechanisms in SZ by analyzing its expression, methylation, and genotypic profiles in PFC and hippocampus (HIPP) post-mortem samples from 30 control (CTL) subjects and 20 individuals with SZ (10 treated with clozapine, SZ-Clz, and 10 without antipsychotic drugs at death, SZ-ApFree). We compared *the NRN1* mRNA expression between groups, measured by qPCR, and methylation levels across three CpG islands, assessed through EpiTYPER. Sparse Partial Least Square Discriminant Analysis identified key CpG units contributing to group differences. We then explored the relationship between *NRN1* methylation and expression, considering the influence of 11 polymorphisms genotyped by qPCR. We found that SZ-Clz had lower *NRN1* mRNA levels in the PFC than SZ-ApFree and CTL. SZ-Clz presented distinct methylation patterns across multiple CpG units in both brain regions compared to CTL. In the PFC, the methylation of the CpG units differentiating SZ-Clz from CTL correlated to *NRN1* expression, and the *NRN1*-rs12333117 and *NRN1*-rs2208870 polymorphisms influenced this effect. These findings reveal distinct correlations between *NRN1* epigenetic expression in SZ-Clz and CTL, shaped by genotypic variability. They emphasize region-specific alterations in SZ and underscore the importance of integrative approaches for a better understanding of the role of candidate genes in SZ etiology.

KEY WORDS

NRN1, schizophrenia, post-mortem brain tissue, methylation, expression, clozapine

1. BACKGROUND

Despite schizophrenia (SZ) research progress, central pathophysiological mechanisms, molecular diagnostics, or precise biomarkers persist unclear. SZ is widely acknowledged to have a high ~80% heritability (1,2), with a complex polygenic architecture involving numerous genetic variants (3). Large-scale genome-wide association studies (GWAS) have identified around 270 loci, many related to synaptic plasticity genes (4). However, these variants incompletely explain total heritability, with most not directly affecting protein structure but notably enriched for variants affecting DNA methylation, gene expression, and gene splicing in the human brain during different developmental stages (5). Together, these findings suggest that both genetic factors and epigenetic mechanisms regulating gene expression contribute to the developmental origins of SZ.

Among studies on epigenetic mechanisms, DNA methylation has been extensively researched, particularly in promoter regions for transcriptional repression, but it also regulates gene expression in distal elements like enhancers, exonic regions, and gene bodies, either inhibiting or activating expression based on genomic context (6). In the brain, methylation plays a twofold role: during development, the balance between methylation of germline-specific genes suppresses pluripotency, and demethylation of neuron-specific genes is key to facilitate neuronal specialization; in the mature brain, methylation changes can be induced by neuronal activity, contributing to complex cognitive processes such as learning and memory (7,8).

Methylation and gene expression studies in SZ, often performed utilizing prefrontal cortex (PFC) or hippocampus (HIPP) tissues, have shown distinct methylation patterns in genes critical for neuron development, synaptogenesis, and synaptic plasticity (9), as well as differential expression of genes related to immune response and inflammation, mitochondrial energy metabolism, myeloid leukocyte activation, cytoskeletal proteins, ion transport regulation, neurite outgrowth, and synaptic plasticity (10). Both types of studies have also suggested that these differences may be region and even cell-specific, with distinct genes implicated accordingly (11). Moreover, considering the impact of methylation on gene expression, numerous studies have correlated promoter hypomethylation of genes related to dopaminergic (*DRD2*, *DRD3*, *COMT*), GABAergic (*GAD1*), serotonergic (*HTR2A*), oligodendrocyte (*SOX10*), and synaptic plasticity (*RELN*) pathways in brain samples from patients with SZ with increased gene expression (12–15).

The above-mentioned studies collectively indicate that exploring methylation's influence on expression in genes involved in brain development and synaptic plasticity might be key to understanding the biological underpinnings of SZ. The Neuritin1 gene (*NRN1*) exemplifies this, as it supports neuronal progenitors and differentiated neurons during early development (16), promotes the growth and stabilization of axonal and dendritic arbors, and facilitates synapse maturation in later development (17), while it also participates in adult

brain synaptic plasticity, particularly in the PFC and the HIPP (18,19). Although *NRN1* has received less attention than traditional GWAS candidate genes, its chromosomal region is linked to an SZ subtype marked by cognitive deficits (20) and genetic association studies have related *NRN1* variants to the risk for SZ, cognitive deficits, early onset, and changes in brain structure and function (21–24). Additionally, two studies comparing patients with SZ to control subjects (CTL) have pinpointed *NRN1*, firstly, as one of the most differentially methylated genes in the PFC in SZ, and secondly, as one of the differentially expressed genes significantly contributing to patient differentiation according to transcriptomic clustering (25,26). Also, various cellular and animal models have shown that *NRN1* expression changes directly impact brain function and that antipsychotic treatments can induce such changes. In animal models, increased Nnr1 expression has been linked to better cognitive performance (27), improved recovery after ischemia-reperfusion injury (28), and protective effects in traumatic brain injury (29). Similarly, Nrn1 expression responds to neurotherapeutic agents like electroconvulsive therapy and fluoxetine through epigenetic pathways involving histone deacetylation (30,31). These findings suggest that modulating the *NRN1* expression could improve SZ symptoms and highlight its potential as a therapeutic target.

Among individuals with SZ undergoing antipsychotic therapy, special attention is given to those with a treatment-resistant profile, which affects about one-third of cases and significantly lowers their quality of life (32). Clozapine, the first atypical antipsychotic, has shown effectiveness in this group (33). It binds to various receptors (34), including dopamine, serotonin, histamine, muscarinic, and adrenergic receptors. Although the exact molecular pathways critical to its effectiveness are unclear, animal studies suggest that clozapine improves behavioral outcomes by modulating genes related to cholesterol metabolism, GABAergic function, cell cycle control, neurotrophins, and synaptic plasticity through reducing methylation or producing histone modifications (35). Meanwhile, human studies are constrained by limited access to post-mortem brain samples and the infrequent use of this treatment among patients. One study using publicly available SZ transcriptomic data found that three genes (*GCLM*, *ZNF652*, *GYPC*) and four pathways involved in protein trafficking, neuronal migration, brain development, and synaptic function were consistently differentially expressed in clozapine-treated patients compared to those on other medications (36).

Overall, current data highlights the role of genetic variability and gene expression regulatory mechanisms, such as methylation, in developmental abnormalities and the brain's response to stimuli, contributing to SZ pathophysiology. Antipsychotics seem to modulate these epigenetic marks, suggesting new therapeutic targets. Therefore, this study aimed to investigate expression, methylation, and genotype variability of *NRN1*,

a synaptic plasticity gene linked to SZ and sensitive to neurotherapeutic agents, in post-mortem PFC and HIPP samples from CTL and patients, considering antipsychotic treatment as a key factor.

2. METHODS

2.1. Post-mortem human brain samples description

Human post-mortem brain samples were collected at the Basque Institute of Legal Medicine (Bilbao, Spain), following research and ethics committee guidelines (Law 14/2007 and RD 1716/2011). It comprised 50 subjects, 20 with an ante-mortem diagnosis of SZ and 30 CTL. The diagnosis was performed by a qualified psychiatrist according to DSM-IV or ICD-10 criteria, as recorded in subjects' medical records. Control subjects had no evidence of any psychiatric disorder in their medical records. Exclusion criteria for both groups included neurological conditions and substance abuse. Post-mortem brain samples from the dorsolateral PFC (Brodmann's area 9) and the HIPP, excluding white matter, were dissected at the time of autopsy and immediately stored at −80°C until posterior analyses. Blood samples of those subjects were used for toxicological screening for antidepressants, antipsychotics, psychotropic drugs, and ethanol performed at the National Institute of Toxicology (Madrid, Spain). For CTL, selection ensured the absence of antidepressants or antipsychotic drugs at the time of death. Finally, both groups were selected to be matched by sex, age, and post-mortem interval (PMI), referring to the time elapsed between the death and the autopsy. Subjects with SZ were subsequently divided according to the presence of clozapine (SZ-Clz) or the absence of antipsychotic medication (SZ-ApFree) in the blood at the time of death. The summary of demographics and sample quality is shown in **Table 1** (see **Table S1** for individual details).

Table 1. Sample characteristics of control subjects (CTL) and individuals diagnosed with schizophrenia (SZ), categorized based on the presence of clozapine (SZ-Clz) or absence of antipsychotic medication (SZ-ApFree) in the blood at the time of death. Demographic information includes sex with female / male (F/M) count and frequency (%) for female category, age (in years), and cause of death. Assessment of post-mortem sample quality encompasses pH and post-mortem interval (PMI, in hours). All the quantitative variables are expressed as mean (standard deviation), while all the qualitative variables are presented as count (percentage). N=natural, O=others (including accidents and homicides), S=suicide.

	CTL (n=30)	SZ-ApFree (n=10)	SZ-Clz (n=10)	χ^2 / U	p-value
Sex (F/M)	10 (33.3%) / 20	3 (30.0%) / 7	4 (40.0%) / 6	0.238	0.888
Age	44.03 (12.10)	46.20 (10.14)	48 (14.90)	0.804	0.658
Death cause (N/O/S) [a]	11 (37%) / 19 (63%) / 0	5 (50%) / 3 (30%) / 2	4 (40%) / 1 (10%) / 5	2.397	0.302
PMI	14.13 (6.65)	16.00 (4.81)	19.20 (4.13)	5.589	0.061
pH	6.37 (0.35)	6.44 (0.28)	6.38 (0.29)	0.920	0.631

[a] This analysis compares SZ-Free with SZ-Clz, as CTL did not die by suicide according to the inclusion criteria.

[b] Data for pH was available for 27 CTL, 9 SZ-Clz and 6 SZ-ApFree.

2.2. Expression analyses

Total RNA was extracted using the NucleoSpin RNA Plus Kit (Cultek). RNA integrity and purity were analyzed by agarose gel electrophoresis and spectrophotometry using a NanoDrop. Reverse transcription reaction was performed with a maximum of 1µg of RNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche) with random hexamer oligonucleotides. For quantitative PCR (qPCR) quantification, 1µL of the reverse transcription reaction was added to the qPCR reaction using JumpStart Taq ReadyMix for quantitative PCR (Sigma). qPCR reaction was preceded with an initial denaturation step at 94ºC for 5 minutes followed by 45 cycles of: denaturation at 94ºC for 15s, annealing at 51ºC for 20s, and extension at 72ºC for 30s. The fluorescent signal resulting from the degradation of the TaqMan probe (6xFAM/Black Hole Quencher1) was read at the end of the amplification phase. The primer sequences and probes are described in **Table S2**.

2.3. DNA extraction

Genomic DNA was extracted from post-mortem brain samples using the Quick-DNA Miniprep Plus Kit (Zymo Research) and used for methylation and genotyping analyses.

2.4. Methylation

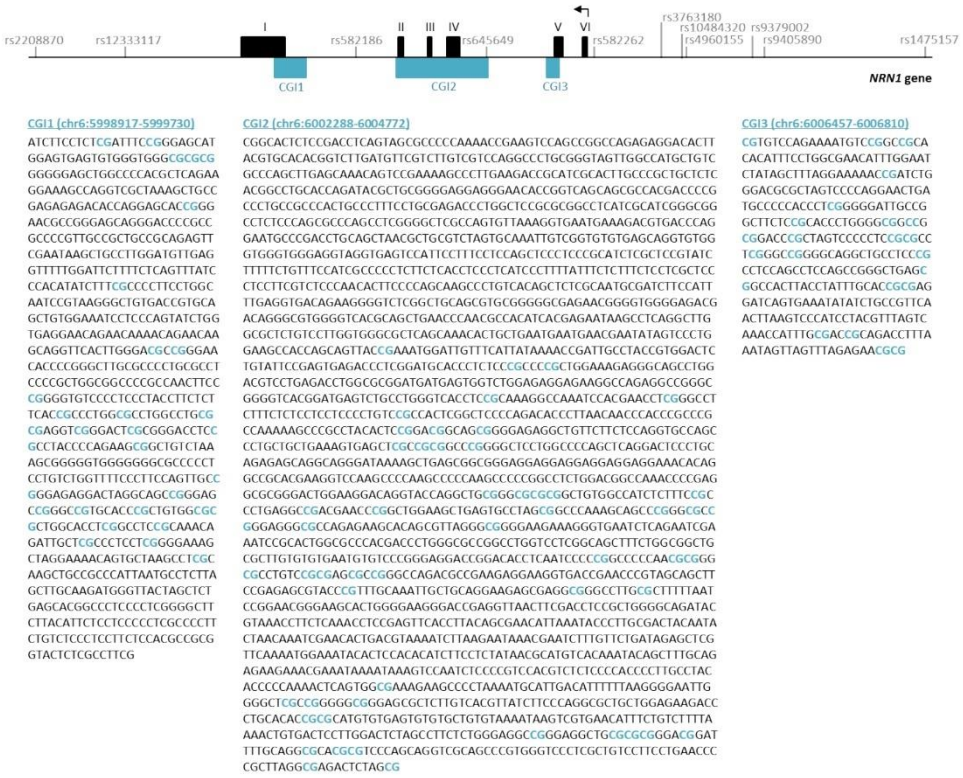
Methylation analyses targeted the CpGs along the three CpG islands (CGI) spanning the *NRN1* gene (CGI1 chr6:5998917-5999730, CGI2 chr6:6002288-6004772 and CGI3 chr6:6006457-6006810, reference genome UCSC-GRC38/hg38). Samples were analyzed using the EpiTYPER method (Agena Bioscience) at the Centro Nacional de Genotipado (CEGEN, http://usc.es/cegen/). This method examines CpG sites within amplicons ranging from 200 to 600 base pairs, being especially well-suited for extensive endeavors aimed at investigating specific regions. In our case, six amplicons were designed using Agena's EpiDesigner software to amplify these regions of interest (**Table S3**).

The methylation values can range between 0 and 1, being 0 the value corresponding to a 0% methylation state and 1 the value associated with a 100% methylated state. If CpG sites are too close together, their methylation levels cannot be determined independently, so the methylation status of a fragment containing multiple sites is measured, reflecting the average methylation of all CpG sites within that fragment. Accordingly, we will refer to CpG units, which can comprise one or more CpG sites. Each CpG unit was denoted numerically based on its 5' to 3' genomic position on the forward strand sequence.

The methylation quality control protocol implemented by CEGEN involved excluding units deviating by over 10% from the established commercial controls (HMc, 100% methylated, and LMc, 0% methylated), as well as excluding the samples with standard deviation variances between replicas exceeding 10%. Additionally, we eliminated units and subjects with more than 35% missing values and replaced outlier methylation values with the maximum for z-scores ≥ 3 and the minimum for z-scores ≤ -3. In all, we have analyzed 63 CpG units for the PFC and 57 CpG units for the HIPP along three CGIs within the *NRN1* region (see the details in **Figure 1** and **Table S4**).

2.5. Genotyping

We genotyped eleven SNPs at the *NRN1* gene (6p25.1) at the National Genotyping Center (CEGEN, http://usc.es/cegen/) that were chosen to cover the genomic sequence and approximately 10 kb upstream and downstream. The optimal set of SNPs, which contained the maximum information about surrounding variants, was selected using the SYSNPs tool (http://www.sysnps.org/) with a minor allele frequency (MAF) > 5%, utilizing the pairwise tagging option ($r^2 \geq 0.8$). Additionally, we included SNPs previously associated with SZ in the study by Chandler et al. (2010). All these SNPs have also been genotyped in other studies conducted by our group (21,21,23,37). The genotyping call rate was 100%, with minor allele frequencies closely matching those of the EUR population in the 1000 Genomes Project (**Table 2**). Genotype frequencies for both groups were in Hardy-Weinberg equilibrium (PLINK v.1.09, (38)).


rs2208870
rs12333117
I
rs582186
II
III
IV
rs645649
V
VI
rs582262
rs3763180
rs10484320
rs4960155
rs9379002
rs9405890
rs1475157
CGI1
CGI2
CGI3
NRN1 gene
CGI1 (chr6:5998917-5999730)
ATCTTCCTCTCGATTTCCGGGAGCAT
GGAGTGAGTGTGGGTGGGCGCGCGG
GGGGGAGCTGGCCCCACGCTCAGAA
GGAAAGCCAGGTCGCTAAAGCTGCC
GAGAGAGACACCAGGAGCACCGGG
AACGCCGGGAGCAGGGACCCCGCC
GCCCCGTTGCCGCTGCCGCAGAGTT
CGAATAAGCTGCCTTGGATGTTGAG
GTTTTTGGATTCTTTTCTCAGTTTATC
CCACATATCTTTCGCCCCTTCCTGGC
AATCCGTAAGGGCTGTGACCGTGCA
GCTGTGGAAATCCTCCCAGTATCTGG
TGAGGAACAGAACAAAACAGAACAA
GCAGGTTCACTTGGGACGCCGGGAA
CACCCCGGGCTTGCGCCCCTGCGCCT
CCCCGCTGGCGGCCCCGCCAACTTCC
CGGGGTGTCCCCTCCCTACCTTCTCT
TCACCGCCCTGGCGCCTGGCCTGCG
CGAGGTCGGGACTCGCGGGACCTCC
GCCTACCCCAGAAGCGGCTGTCTAA
AGCGGGGGTGGGGGGGCGCCCCCT
CCTGTCTGGTTTTCCCTTCCAGTTGCC
GGGAGAGGACTAGGCAGCCGGGAG
CCGGGCCGTGCACCCGCTGTGGCGC
GCTGGCACCTCGGCCTCCGCAAACA
GATTGCTCGCCCTCCTCGGGAAAG
CTAGGAAAACAGTGCTAAGCCTCGC
AAGCTGCCGCCCATTAATGCCTCTTA
GCTTGCAAGATGGGTTACTAGCTCT
GAGCACGGCCCTCCCCTCGGGGCTT
CTTACATTCTCCTCCCCCTCGCCCCTT
CTGTCTCCCTCCTTCTCCACGCCGCG
GTACTCTCGCCTTCG

CGI2 (chr6:6002288-6004772)
CGGCACTCTCCGACCTCAGTAGCGCCCCCAAAACCGAAGTCCAGCCGGCCAGAGAGGACACTT
ACGTGCACACGGTCTTGATGTTCGTCTTGTCGTCCAGGCCCTGCGGGTAGTTGGCCATGCTGTC
GCCCAGCTTGAGCAAACAGTCCGAAAAGCCCTTGAAGACCGCATCGCACTTGCCCGCTGCTCTC
ACGGCCTGCACCAGATACGCTGCGGGGAGGAGGGAACACCGGTCAGCAGCGCCACGACCCCG
CCCTGCCGCCCACTGCCCTTTCCTGCGAGACCCTGGCTCCGCGCGGCCTCATCGCATCGGGCGG
CCTCTCCCAGCGCCCAGCCTCGGGGCTCGCCAGTGTTAAAGGTGAATGAAAGACGTGACCCAG
GAATGCCCGACCTGCAGCTAACGCTGCGTCTAGTGCAAATTGTCGGTGTGTGAGCAGGTGTGG
GTGGGTGGGAGGTAGGTGAGTCCATTCCTTTCCTCCAGCTCCCTCCCGCATCTCGCTCCGTATC
TTTTTCTGTTTCCATCGCCCCCTCTTCTCACCTCCCTCATCCCTTTTATTTCTCTTTCTCCTCGCTCC
CTCCTTCGTCTCCCAACACTTCCCCAGCAAGCCCTGTCACAGCTCTCGCAATGCGATCTTCCATT
TTGAGGTGACAGAAGGGGTCTCGGCTGCAGCGTGCGGGGGCGAGAACGGGGTGGGGAGACG
ACAGGGCGTGGGGTCACGCAGCTGAACCCAACGCCACATCACGAGAATAAGCCTCAGGCTTG
GCGCTCTGTCCTTGGTGGGCGCTCAGCAAACACTGCTGAATGAATGAACGAATATAGTCCCTG
GAAGCCACCAGCAGTTACCGAAATGGATTGTTTCATTATAAAACCGATTGCCTACCGTGGACTC
TGTATTCCGAGTGAGACCCTCGGATGCACCCTCTCCCGCCCCGCTGGAAAGAGGGCAGCCTGG
ACGTCCTGAGACCTGGCGCGGATGATGAGTGGTCTGGAGAGGAGAAGGCCAGAGGCCGGGC
GGGGTCACGGATGAGTCTGCCTGGGTCACCTCCGCAAAGGCCAAATCCACGAACCTCGGGCCT
CTTTCTCTCCTCCTCCCCTGTCCGCCACTCGGCTCCCCAGACACCCTTAACAACCCACCGCCCG
CCAAAAAGCCCGCCTACACTCCGGACGGCAGCGGGGAGAGGCTGTTCTTCTCCAGGTGCCAGC
CCTGCTGCTGAAAGTGAGCTCGCCGCGGCCCGGGGCTCCTGGCCCCAGCTCAGGACTCCCTGC
AGAGAGCAGGCAGGGATAAAAGCTGAGCGGCGGGAGGAGGAGGAGGAGGAGGAAACACAG
GCCGCACGAAGGTCCAAGCCCCAAGCCCCCAAGCCCCCGGCCTCTGGACGGCCAAACCCCGAG
GCGCGGGACTGGAAGGACAGGTACCAGGCTGCGGGCGCGCGGCTGTGGCCATCTCTTTCCGC
CCTGAGGCCGACGAACCCGGCTGGAAGCTGAGTGCCTAGCGGCCCAAAGCAGCCCGGGCGCCC
GGGAGGGCGCCAGAGAAGCACAGCGTTAGGGCGGGGAAGAAAGGGTGAATCTCAGAATCGA
AATCCGCACTGGCGCCCACGACCCTGGGCGCCGGCCTGGTCCTCGGCAGCTTTCTGGCGGCTG
CGCTTGTGTGTGAATGTGTCCCGGGAGGACCGGACACCTCAATCCCCCGGCCCCCAACGCGGG
CGCCTGTCCGCGAGCGCCGGGCCAGACGCCGAAGAGGAAGGTGACCGAACCCGTAGCAGCTT
CCGAGAGCGTACCCGTTTGCAAATTGCTGCAGGAAGAGCGAGGCGGGCCTTGCGCTTTTTAAT
CCGGAACGGGAAGCACTGGGGAAGGGACCGAGGTTAACTTCGACCTCCGCTGGGGCAGATAC
GTAAACCTTCTCAAACCTCCGAGTTCACCTTACAGCGAACATTAAATACCCTTGCGACTACAATA
CTAACAAATCGAACACTGACGTAAAATCTTAAGAATAAACGAATCTTTGTTCTGATAGAGCTCG
TTCAAAATGGAAATACACTCCACACATCTTCCTCTATAACGCATGTCACAAATACAGCTTTGCAG
AGAAGAAACGAAATAAAATAAAGTCCAATCTCCCCGTCCACGTCTCTCCCCACCCCTTGCCTAC
ACCCCCAAAACTCAGTGGCGAAAGAAGCCCCTAAAATGCATTGACATTTTTTAAGGGGAATTG
GGGCTCGCCGGGGGCGGGAGCGCTCTTGTCACGTTATCTTCCCAGGCGCTGCTGGAGAAGACC
CTGCACACCGCGCATGTGTGAGTGTGTGCTGTGTAAAATAAGTCGTGAACATTTCTGTCTTTTA
AAACTGTGACTCCTTGGACTCTAGCCTTCTCTGGGAGGCCGGGAGGCTGCGCGCGGGACGGAT
TTTGCAGGCGCACGCGTCCCAGCAGGTCGCAGCCCGTGGGTCCCTCGCTGTCCTTCCTGAACCC
CGCTTAGGCGAGACTCTAGCG

CGI3 (chr6:6006457-6006810)
CGTGTCCAGAAAATGTCCGGCCGCA
CACATTTCCTGGCGAACATTTGGAAT
CTATAGCTTTAGGAAAAACCGATCTG
GGACGCGCTAGTCCCCAGGAACTGA
TGCCCCCACCCTCGGGGGATTGCCG
GCTTCTCCGCACCCTGGGGCGGCCG
CGGACCCGCTAGTCCCCTCCGCGCC
TCGGGCCGGGCAGGCTGCCTCCCG
CCTCCAGCCTCCAGCCGGGCTGAGC
GGCCACTTACCTATTTGCACCGCGAG
GATCAGTGAAATATATCTGCCGTTCA
ACTTAAGTCCCATCCTACGTTTAGTC
AAACCATTTGCGACCGCAGACCTTTA
AATAGTTAGTTTAGAGAACGCG

Figure 1. Schematic representation of the *NRN1* gene (NCBI36/hg18) with exonic (in black) and CpG island (CGI, in blue) regions, as well as single nucleotide polymorphisms (SNPs) included in the study according to the human genome browser (http://genome.ucsc.edu). Positions of the analyzed CpG sites in the DNA sequence are indicated below (in bold blue).

Table 2. Information regarding *NRN1* SNPs included in this study. The table contains the dbSNP number, chromosome location (Chr Pos), alleles, and minor allele frequency (MAF) for each SNP based on the UCSC Genome Browser on Human (GRCh38/hg38). Additionally, MAF and genotypic count and frequency (%) are listed separately for control subjects (CTL) and subjects with schizophrenia (SZ), categorized based on the presence of clozapine (SZ-Clz) or absence of antipsychotic medication (SZ-ApFree) in the blood at the time of death. Due to low frequency in some genotypes, frequencies shown and subsequent analyses were conducted with all SNPs dichotomized (homozygous and heterozygous for the minor allele versus homozygous for the major allele).

dbSNP	Chr Pos	Allele	MAF_{1000G}	MAF_{sample}	CTL freq	SZ-ApFree (n=10)	SZ-Clz (n=10)
rs2208870	6:5992257	G/A	0.34	0.33	16 (53.3%) / 14	4 (40.0%) / 6	6 (60.0%) / 4
rs12333117	6:5994759	T/C	0.40	0.41	16 (53.3%) / 14	8 (80.0%) / 2	8 (80.0%) / 2
rs582186	6:6001148	A/G	0.38	0.43	20 (66.7%) / 10	6 (60.0%) / 4	6 (60.0%) / 4
rs645649	6:6004726	C/G	0.36	0.34	18 (60.0%) / 12	5 (50.0%) / 5	5 (50.0%) / 5
rs582262	6:6007758	C/G	0.30	0.32	18 (60.0%) / 12	6 (60.0%) / 4	4 (40.0%) / 6
rs3763180	6:6009615	T/G	0.46	0.48	22 (73.3%) / 8	7 (70.0%) / 3	7 (70.0%) / 3
rs10484320	6:6010204	T/C	0.22	0.20	10 (33.3%) / 20	2 (20.0%) / 8	2 (20.0%) / 8
rs4960155	6:6010306	C/T	0.49	0.52	22 (73.3%) / 8	8 (80.0%) / 2	8 (80.0%) / 2
rs9379002	6:6012158	G/T	0.29	0.28	14 (46.7%) / 16	7 (70.0%) / 3	5 (50.0%) / 5
rs9405890	6:6012488	C/T	0.31	0.26	12 (40.0%) / 18	3 (30.0%) / 7	4 (40.0%) / 6
rs1475157	6:6016936	G/A	0.17	0.19	10 (33.3%) / 20	5 (50.0%) / 5	3 (30.0%) / 7

2.4. Statistical analysis

2.4.1. Sample characteristics

For mean comparisons between cases and controls concerning sociodemographic and clinical characteristics as well as biological quality of the sample, Wilcoxon Rank Sum Test (for quantitative variables) and chi-square tests (for qualitative variables) were used.

2.4.2. Expression analyses

Gene expression levels were determined with Delta Ct method (39), relativizing each gene expression to GAPDH levels. For the analysis, confounding variables (sex, age, death cause, PMI, and pH) were regressed from gene expression. Subsequently, a multifactorial ANOVA analysis was conducted followed by Tukey's

honest significance test, used for multiple comparisons of means between groups (CTL, SZ-ApFree and SZ-Clz).

2.4.3. Methylation analyses

For methylation analyses, we assembled a subset consisting of 10 individuals each from the CTL, SZ-Clz, and SZ-ApFree groups, ensuring no significant differences with the initial sample in either demographic factors or post-mortem sample quality, as well as in *NRN1* expression across both regions (**Table S4** and **Figure S1**). Prior to analyzing methylation data, the confounding variables (sex, age, illness duration, and clozapine dosage) were regressed from each predictor methylation variable (CpG units).

To overcome the high intercorrelations among CpG units and the resulting multicollinearity (**Table S5**), we employed sparse Partial Least Squares-Discriminant Analysis (sPLS-DA), a method that simultaneously performs dimensionality reduction, feature selection, and classification to examine methylation differences between groups. Similar techniques, such as Principal Component Analysis (PCA), have been used in methylation studies to reduce high-dimensional genomic or locus-specific data into principal components that capture overall variance among CpG units in an unsupervised manner (40,41). In contrast, sPLS-DA is a supervised method that identifies latent components (LCs) by maximizing the covariance between the most relevant CpG units and group labels, enabling the discovery of methylation patterns associated with specific groups and enhancing the clinical significance of the findings.

All the analyses were based on the mixOmics protocol as implemented in the R package (42). To address the challenge posed by the small sample size in our study, we applied Leave-One-Out Cross-Validation (LOOCV) at multiple stages of the modelling process to calculate classification error. LOOCV is particularly well-suited to small datasets as it maximizes each data point's use by treating each individual sample as a test set once, while training the model on the remaining data. First, LOOCV was used to determine the optimal number of LCs by evaluating the classification error across different component configurations. To further optimize the model, we applied the LASSO penalty in combination with LOOCV to select the most relevant CpG units. After determining the optimal number of LCs and CpG units, we used LOOCV to evaluate the performance of the final sPLS-DA model. We interpreted each CpG unit within an LC in two main ways: first, through its coefficient or loading value, which indicates its importance in distinguishing between groups, and second, by assessing the contribution of the CpG, which identifies the group that shows higher methylation values. Additionally, to validate the reproducibility of the CpG signature captured by the LCs, we performed a feature stability analysis, which assessed how frequently each CpG site was selected across multiple iterations of the model.

We selected the most informative LC using two criteria: i) first, following the sPLS-DA methodology, where the first component captures the maximum covariance between features and the outcome, which we further validated by comparing LC scores between groups using the Mann-Whitney U test; and ii) second, based on the stability of CpG units within each LC, as subsequent analyses focused on examining methylation patterns defined by these CpG units.

To further characterize the CpG signature captured by the LCs and find methylation patterns, following other studies with similar approaches (43,44), we computed two means: one grouping CpG units showing greater methylation in the SZ group (referred as “positive mean”); and another grouping CpG units with lower methylation in the SZ group (referred as “negative mean”). To validate the robustness of these patterns and ensure that the observed differences were not merely the result of a data-driven process, we also utilized the Mann-Whitney U test to statistically compare these means between the two groups.

2.4.4. Expression-methylation correlates

We conducted four linear regression analyses using the expression residuals of the HIPP or the PFC as described in section *2.4.2 Expression Analyses*. For each brain region, we tested two models: one with overall methylation variation (LC1) as the predictor and another with positive and negative mean methylation values as predictors.

2.4.5. Genotypic analyses

We conducted four stepwise regression analyses using the expression residuals of the HIPP or the PFC as described in section *2.4.2 Expression Analyses*. For each brain region, we tested two models: one with overall methylation variation (LC1) with 11 *NRN1* genetic variants (SNPs) as the predictors and another with positive and negative mean methylation values with 11 *NRN1* genetic variants (SNPs) as predictors.

3. RESULTS

3.1. Sample description.

There were no differences in sociodemographic nor in the quality of the biological sample between groups, as they were matched for sex, age and PMI as shown in **Table 1**.

3.2. *NRN1* mRNA expression differences between groups.

We found group differences in the PFC (F=10.160 and p=0.002) but not in the HIPP (F=2.576 and p=0.088). Tukey’s post-hoc analysis showed that SZ-Clz had significantly reduced *NRN1* mRNA expression levels in the

PFC compared to CTL (Δ-means=-7.10 and p-adj=0.007) and SZ-ApFree (Δ-means=-8.85 and p-adj=0.005) (**Figure 2**).

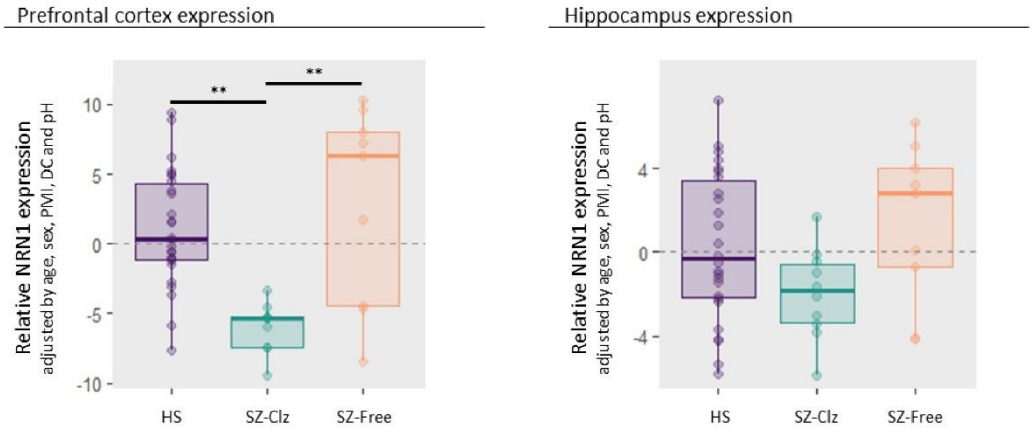


Figure 2. Boxplots representing NRN1 expression levels ± 2 standard errors (SE) depicted according to the group, control subjects (CTL), schizophrenia patients (SZ) categorized based on the presence of clozapine (SZ-Clz) or absence of antipsychotic medication (SZ-ApFree) in the blood at the time of death, for both brain regions: **A)** Prefrontal cortex (n=27, 9, and 9 respectively), and **B)** hippocampus (n=26, 9, and 10 respectively). The NRN1 expression was relativized to GAPDH, and the values expressed are the residuals (adjusted by sex, age, post-mortem interval (PMI), death cause (DC) and pH). Adjusted p-values from Tukey's test are represented as * p<0.05, ** p<0.01, *** p<0.001.

To validate the findings concerning *NRN1* expression we also analyzed the expression of two other synapse-related genes well described to be sensitive to clozapine treatment, *UMHK1* and *BDNF* (45). Like *NRN1*, significant group differences emerged within the PFC for both *UMHK1* (F=5.73, p=0.006) and *BDNF* (F=8.993, p<0.001), while not in the HIPP. Tukey's post-hoc analysis revealed that clozapine treatment was markedly associated with a decrease of *UMHK1* mRNA expression levels in the PFC compared to both CTL (Δ-means=-2.589, p-adj=0.032) and SZ-ApFree (Δ-means=-3.968, p-adj=0.005), while *BDNF* expression levels were notably higher compared to CTL (Δ-means=1.307, p-adj < 0.001) (**Figure S2**).

3.3. *NRN1* methylation differences between groups

Initially, our analysis encompassed all three groups, but we observed a limited ability to identify a specific linear combination of CpG units from either the PFC or the HIPP that could effectively differentiate between the three groups (balanced error for the model incorporating CpG units from the PFC=0.74 and from the HIPP=0.66). Furthermore, upon projecting the samples into the two LC spaces determined by the respective models, we observed a significant overlap between CTL and SZ-ApFree, but a clear distinction of SZ-Clz in both regions (**Figure S3 and Figure S4**).

Subsequently, we generated two separate models for each region, focusing on the CTL and SZ-Clz groups. In the PFC, three LCs explained 12%, 17%, and 10% of the methylation variance, respectively, effectively distinguishing the groups (balanced error=0.28) (**Figure 3A**). Similarly, in the HIPP, two LCs explained 34% and 22% of the variance, achieving the same level of separation (balanced error=0.28) (**Figure 3C**). Due to the sPLS-DA methodology, LC1 was the most influential in differentiating the groups in both regions. This was further confirmed by significant differences observed in PFC-LC1 ($W=76$, $p<0.001$) and HIPP-LC1 ($W=71$, $p=0.005$), while the other LCs did not reach statistical significance, neither in the PFC (LC2: $W=62$, $p=0.06$; LC3: $W=51$, $p=0.38$; **Figure 3B**) nor in the HIPP (LC2: $W=61$, $p=0.08$; **Figure 3D**). Additionally, PFC-LC1 and HIPP-LC1 exhibited greater feature stability, with at least 88% of CpG units showing high stability (>85%), compared to less than 50% in the other LCs (**Table S6**). Accordingly, subsequent analyses of methylation patterns among the CpG units focused on LC1 in both regions.

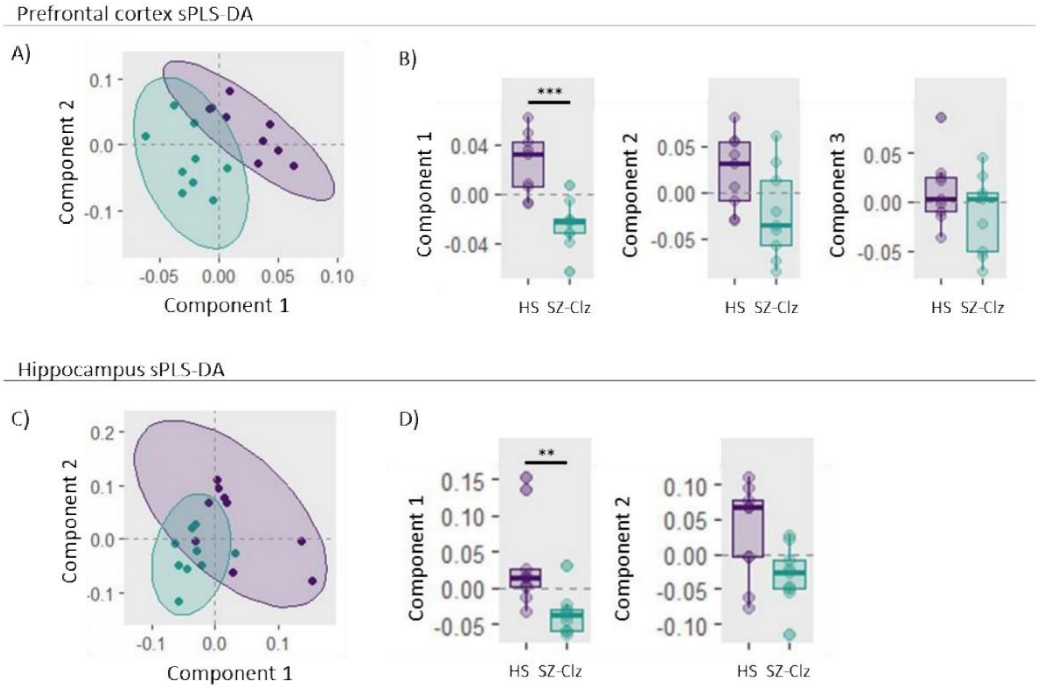
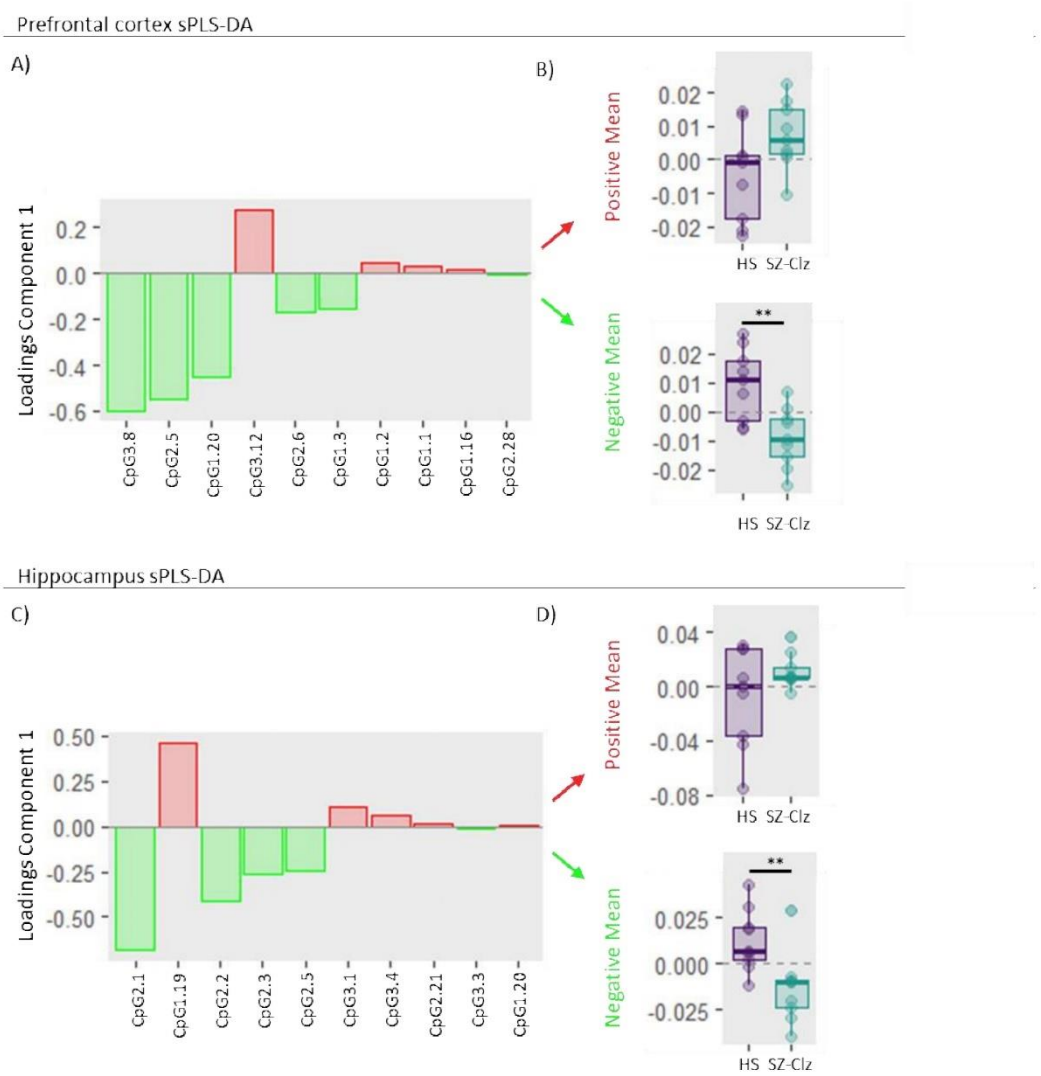


Figure 3. A and C) Scatter plot illustrating the distribution of samples projected into the space defined by the first two components derived from sparse Partial Least Square Discriminant Analysis (sPLS-DA) using *NRN1* methylation values from the hippocampus (HIPP). Each sample class is enclosed within 95% confidence ellipses. **B and D)** Boxplots representing methylation scores for the first and second latent components (LC) ± 2 standard errors (SE) depicted according to the group. Control subjects (CTL) are depicted in purple while schizophrenia patients treated with clozapine (SZ-Clz) are shown in blue. * p<0.05, ** p<0.01, *** p<0.001.

In both brain regions, LC1 included 10 CpG units with varying importance in distinguishing between classes. CpG units with positive weights, represented by red bars, exhibited higher methylation levels in SZ-Clz subjects compared to CTL, while those with negative weights, shown in green, exhibited lower methylation levels in SZ-Clz subjects (**Figure 4A and C**). In both regions, the mean of the CpG units in LC1 with reduced methylation in SZ-Clz subjects compared to CTL significantly differed between groups ("PFC negative average": CpG3.8, CpG2.5, CpG1.20, CpG2.6, CpG1.3, and CpG2.28, W=69, p=0.01; "HIPP negative average": CpG2.1, CpG2.2, CpG2.3, CpG2.5, and CpG3.3, W=70, p=0.008), suggesting that the observed differences are not merely data-driven artifacts. In contrast, the mean of the CpG units in LC1 with higher methylation in SZ-Clz subjects compared to CTL did not show significant differences in either region ("PFC positive average": CpG3.12, CpG1.2, CpG1.1, and CpG1.16, W=18, p=0.05, **Figure 4B**; "HIPP positive average": CpG1.19, CpG3.1, CpG3.4, CpG2.21, and CpG1.20, W=31, p=0.44, **Figure 4D**).



246 **Figure 4. A and C)** Bar plots illustrating the loading weights for each CpG unit in component 1. Red bars indicate higher
247 methylation in patients with schizophrenia (SZ) treated with clozapine (SZ-Clz), while green bars represent lower
248 methylation in SZ-Clz compared to control subjects (CTL). **B and D)** Boxplots representing the mean methylation values,
249 computed based on whether the CpGs exhibited higher (positive mean) or lower (negative mean) methylation levels in SZ-
250 Clz compared to CTL, ±2 standard errors (SE) depicted according to the group. SZ-Clz are shown in blue and CTL in
251 purple. * p<0.05, ** p<0.01, *** p<0.001.

3.3. *NRN1* expression methylation correlates

We observed a significant correlation between LC1 scores derived from the PFC sPLS-DA model and *NRN1* gene expression in the corresponding brain region across the sample including both SZ-Clz and CTL groups (standardized β=0.753, p<0.001, adj-R^2=0.536). When we further analyzed the CpG units based on their methylation status in the SZ-Clz group, the correlation remained significant for the "negative mean" values (standardized β=0.715, p=0.002, adj-R^2=0.477), while the "positive mean" values were not associated with NRN1 expression. These results indicate that lower LC1 scores or "negative mean" values are linked to a reduced NRN1 expression (**Figure 5** and **Figure S5**). Conversely, we did not find any correlation between NRN1 expression and methylation in the HIPP.

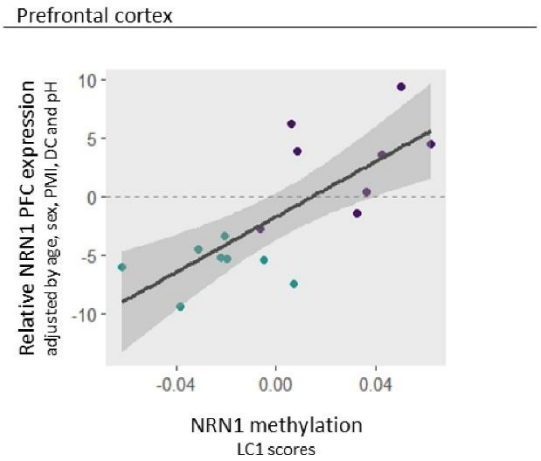


Figure 5. Scatter plot illustrating the correlation between latent component 1 (LC1) scores derived from the sparse Partial Least Square Discriminant Analysis (sPLS-DA) of the prefrontal cortex (PFC) and *NRN1* gene expression levels in the same region. *NRN1* gene expression levels are shown with ±2 standard errors (SE). The expression of *NRN1* was normalized to GAPDH, and the values are presented as residuals, adjusted for sex, age, post-mortem interval (PMI), cause of death (DC), and pH. The sample includes both schizophrenia patients treated with clozapine (SZ-Clz) and control subjects (CTL), with dots colored blue for SZ-Clz patients and purple for CTL.

3.4 *NRN1* genetic variability effects

We found that incorporating two genetic variants, rs2208870 and rs12333117, along with LC1 scores or the "negative mean" values from the PFC sPLS-DA model, enhanced the correlation with *NRN1* gene expression in the corresponding brain region in the sample including SZ-Clz and CTL groups. Adding LC1 scores alongside the two genetic variants significantly enhanced the model's performance compared to using only the methylation variable (model's F=11.3, Adj-R^2=0.673, p=0.001; Δ-R^2=0.137, p of the change=0.048). LC1 scores exhibited a robust association with NRN1 gene expression (standardized β=0.770, p<0.001), while the genetic variants also made significant contributions (standardized β=-0.404, p=0.029 for rs2208870;

standardized β=-0.364, p=0.045 for rs12333117). Similar improvements were observed when incorporating the "negative mean" values alongside the two genetic variants, significantly enhancing the model's performance compared to using only the methylation variable (model's F=12.52, Adj-R^2=0.697, p=0.001; Δ-R^2=0.221, p of the change=0.015). The "negative mean" exhibited a strong association with NRN1 gene expression (standardized β=0.790, p<0.001), while the genetic variants also made notable contributions (standardized β=-0.490, p=0.010 for rs2208870; standardized β=-0.442, p=0.016 for rs12333117). Conversely, we found that incorporating genotypic variability into the methylation metric did not enhance the model's ability to explain *NRN1* expression in the HIPP.

4. Discussion

Given the crucial roles of *NRN1* in brain development and synaptic plasticity, and the significance of these mechanisms in the etiology of SZ, we have investigated the molecular variability of this gene in post-mortem brain samples from patients with SZ and CTL. Our study reveals that *NRN1* is significantly under-expressed in the PFC of patients treated with clozapine and that methylation levels correlate with *NRN1* expression in the same region. In addition, our study shows that incorporating genetic variability data of *NRN1* further shapes this correlation, highlighting the necessity of multi-level approaches to understand the role of candidate genes in SZ etiology.

First, our results showed that patients treated with clozapine exhibited reduced expression of *NRN1* in the PFC as compared to both CTL and Sz-ApFree patients. On the one hand, these differences should be considered in terms of the their functional impact, as *NRN1* expression is crucial as it enhances synaptic transmission by increasing the surface expression of CaV1.2, CaV1.3, and CaV3.3 channels, mediated through the insulin receptor and ERK signaling pathways (46–48). Then, our data provides new evidence suggesting that the reduced NRN1 PFC expression observed in patients with SZ may be specifically associated with, or at least more pronounced under, clozapine treatment, and that such differences could be related to synaptic transmission variability. Despite the scarcity of comparable studies, to further interpretating our findings it is interesting to mention that epigenetic mechanisms have been implicated in the effects of atypical antipsychotic treatment, specifically demonstrating that chronic treatment induces the expression of proteins related to histones deacetylation, such as HDAC2 (49). Particularly, clozapine treatment has been related to widespread promoter demethylation (50). Also, it is remarkable a previous study exploring dorsolateral PFC transcriptome of schizophrenia patients, that identified two molecularly distinct subgroups of patients, one similar to CTL and the other markedly different, with NRN1 being among the down-regulated genes between the latter subgroup and CTL (25). Then, we tried to replicate our results with a larger sample of clozapine-treated patients, but

306 public data was limited and had fewer treated patients than our study (GSE224683, GSE80655, GSE12649).
307 On the other hand, the fact that our study did not reveal the same effect in HIPP, is aligned with previous
308 research showing different anatomical methylation signatures in patients with SZ and CTL when comparing
309 the PFC and the HIPP, possibly due to varying cellular compositions and their corresponding functional
310 specializations (51). This interpretation within the framework of region-specific transcriptome alterations in SZ
311 mirrors the loss-of-function mutations in the *GRIN2A* gene, encoding an NMDA receptor subunit, which
312 significantly increase SZ risk by differentially affecting brain regions and circuits (52,53).

313 Second, we explored the methylation profile of *NRN1* in the two brain regions, but recognizing the complexity
314 of interpreting methylation changes in SZ, in a complementary approach to the global perspective given by the
315 PLS, we advanced to more detailed interpretations to integrate both broad methylation patterns and targeted
316 site-specific analyses. As regards the differences between SZ-Clz and CTL, defined by distinct CpG units in
317 each region, we observed that the component LC1, which best distinguished the two groups, included CpG
318 units with both increased and decreased methylation in patients, indicating that bidirectional changes are a
319 defining feature of the patient methylation profile across both brain regions. However, upon grouping CpG
320 units by the direction of methylation change, we found that the average of those CpG units under-methylated
321 in SZ-Clz was significantly different between groups in both regions, suggesting that lower methylation in SZ-
322 Clz may play a more prominent role. Notably, a methylome-wide study identified a large region
323 (chr6:59992669-6006917) encompassing 29 adjacent CpG sites along the gene body of the *NRN1* as
324 hypomethylated in PFC of SZ patients compared to CTL (26). This region included all CpG sites detected as
325 hypomethylated in our study except for CpG 1.3, hence our findings add to those from Pidsley et al., 2014
326 while also suggesting that the observed hypomethylation may be characteristic of clozapine-treated patients.

327 Third, when exploring the relationship between *NRN1* methylation and expression patterns, we observed that
328 two methylation metrics derived from the PFC model, LC1 and the average of those CpG units under-
329 methylated correlated with *NRN1* expression in this region. Although the novelty of our study limits direct
330 comparison with other *NRN1*-specific results, our findings are consistent with previous research linking DNA
331 methylation to differential gene expression in post-mortem brain samples from individuals with SZ (54).
332 Additionally, it is important to note that some studies have linked clozapine treatment, but not haloperidol or
333 olanzapine, to changes in *BDNF* expression, an upstream regulator of *NRN1*, through the reduction of its
334 promoter methylation (55).

335 Next, to gain further insights into the potential methylation-expression correlates, we identified regulatory
336 elements overlapping with CpG units within PFC LC1 by using the UCSC Genome Browser

(http://genome.ucsc.edu) to access data from the Encyclopedia of DNA Elements (ENCODE) project (56) and the Open Regulatory Annotation (ORegAnno) database (57). The presence of enhancer regions, DNase I hypersensitive sites within these CpG units, and histone marks like H3K27me3, H3K4me1, and H3K36me3 highlights their potential in gene expression control and chromatin accessibility. Upon examining the transcription factor binding sites (TFBS) influenced by these CpGs, we observed that most of the CpGs under-methylated in SZ-Clz potentially altered the binding of numerous transcription factors (TFs). Many of these TF have been previously associated with SZ. For instance, CpG1.3 methylation variability may impact the binding of ELK1, differentially expressed in SZ lymphoblastoid cells (58), FEV, linked to affective disorders (59), and SPDEF, which methylation moderates stress and substance abuse and its expression differs in the brain of patients with autism spectrum disorders and CTL (60). Similarly, CpG2.5 and CpG2.6 could affect the binding of EGR1, a stress response gene genetically associated with SZ (61).

Four, considering that many SZ-associated genetic variants have regulatory functions and that methylation is under local genetic control (62), we integrated genotypic variability in our analysis In the PFC, two variants (rs2208870 and rs12333117) along with LC1 scores or hypomethylated CpG units correlated with *NRN1* expression. HaploReg v4.2 data (63) showed that both variants alter TF binding affinity, with rs12333117 also affecting enhancer and promoter function in multiple tissues, including the brain.

Despite the insights gained into the effects of genetic variability and methylation patterns on *NRN1* expression in SZ, several limitations must be mentioned. Our relatively small sample size, although comparable to other studies, may not capture the full variability of the methylation landscape in SZ, limiting the generalizability of our findings. Additionally, the expression and methylation relationship with antipsychotics is two-way: antipsychotics can modulate methylation and thereby gene expression, but the unique methylation profile of an individual prior to treatment can also affect drug efficacy. Thus, without a group of never-treated patients, we cannot truly separate the effects of the disorder from those of the medication, but finding such post-mortem brain samples is challenging. Moreover, post-mortem samples also introduce variability due to tissue heterogeneity and differences in the cause of death, though we tried to account for this by including this last as a covariate. Finally, our cross-sectional study design limits our ability to assess changes in methylation over time, which is crucial for understanding SZ progression and treatment response. Moreover, establishing causality in such studies remains difficult. Therefore, further research with larger samples, longitudinal design, and advanced multi-omics approaches are needed to fully understand the epigenetic mechanisms underlying SZ.

In conclusion, we found that *NRN1* is significantly under-expressed in the PFC of patients with SZ treated with clozapine, with methylation levels correlating with its expression. Our study underscores distinct *NRN1* epigenetic patterns in the PFC and HIPP, highlighting the brain region specificity of SZ-related alterations. Additionally, incorporating genetic variability enhanced these correlations, emphasizing the importance of considering both genetic and epigenetic factors to fully understand the molecular mechanisms underlying SZ.

6. ACKNOWLEDGEMENTS

This work was supported by: i) Fundación Alicia Koplowitz, ii) Acadèmia de les Ciències Mèdiques i de la Salut de Catalunya i de Balears. This study also received funding provided by: i) the Instituto de Salud Carlos III through a Miguel Servet contract to MF–V (CP20/00072), co-funded by European Regional Development Fund (ERDF)/European Social Fund “Investing in your future”; (i) the Acadèmia de les Ciències Mèdiques i de la Salut de Catalunya i de Balears through a contract to CA-P; iii) the Comissionat per a Universitats i Recerca del DIUE of the Generalitat de Catalunya, Agència de Gestió d’Ajuts Universitaris i de Recerca (AGAUR: 2021SGR1475); iv) the Basque Government (IT1512/22).

7. ETHICAL STATEMENTS

The study adhered to legal and ethical standards, with all samples collected at the Basque Institute of Legal Medicine (Bilbao, Spain) in accordance with Spanish national research policies and ethical guidelines for post-mortem brain studies in effect at the time of collection (Law 14/2007). Additional approval was obtained from the Research Ethics Committees of the Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU) in País Vasco, Spain (RD 1716/2011), and Germanes Hospitalaries in Cataluña, Spain (PR-2016-07).

8. CONFLICT OF INTERESTS

All the authors reported no biomedical financial interests or potential conflicts of interest.

9. AUTHORSHIP CONTRIBUTION STATEMENT

Carmen Almodóvar-Payá: Conceptualization, Data curation, Methodology, Investigation, Formal Analysis, Visualization, Writing – original draft, Writing – review & editing. **Marcos Moreno:** Data Curation, Methodology, Investigation, Formal Analysis, Writing – review & editing. **Maria Guardiola-Ripoll:** Data Curation, Investigation, Writing – review & editing. **Mariona Latorre-Guardia:** Methodology, Formal Analysis, Writing – review & editing. **Benito Morentin:** Data curation, Resources, Writing – review & editing. **Beatriz Garcia-Ruíz:** Investigation, Writing – review & editing. **Edith Pomarol-Clotet:** Resources, Funding acquisition, Writing – review & editing. **Luis F Callado:** Data Curation, Resources, Funding acquisition, Writing – review & editing. **Carme Gallego:** Conceptualization, Investigation, Methodology, Resources, Funding acquisition, Supervision,

Writing – review & editing. **Mar Fatjó-Vilas:** Conceptualization, Methodology, Resources, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

10. BIBLIOGRAPHY

1. Hilker R, Helenius D, Fagerlund B, Skytthe A, Christensen K, Werge TM, et al. Heritability of Schizophrenia and Schizophrenia Spectrum Based on the Nationwide Danish Twin Register. Biological Psychiatry. 2018 Mar;83(6):492–8.
2. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a Complex Trait: Evidence From a Meta-analysis of Twin Studies. Arch Gen Psychiatry. 2003 Dec 1;60(12):1187.
3. Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature. 2009 Aug;460(7256):748–52.
4. Trubetskoy V, Pardiñas AF, Qi T, Panagiotaropoulou G, Awasthi S, Bigdeli TB, et al. Mapping genomic loci implicates genes and synaptic biology in schizophrenia. Nature. 2022 Apr;604(7906):502–8.
5. Takata A, Matsumoto N, Kato T. Genome-wide identification of splicing QTLs in the human brain and their enrichment among schizophrenia-associated loci. Nat Commun. 2017 Feb 27;8(1):14519.
6. Moore LD, Le T, Fan G. DNA Methylation and Its Basic Function. Neuropsychopharmacol. 2013 Jan;38(1):23–38.
7. Halder R, Hennion M, Vidal RO, Shomroni O, Rahman RU, Rajput A, et al. DNA methylation changes in plasticity genes accompany the formation and maintenance of memory. Nat Neurosci. 2016 Jan;19(1):102–10.
8. Sun M an, Sun Z, Wu X, Rajaram V, Keimig D, Lim J, et al. Mammalian Brain Development is Accompanied by a Dramatic Increase in Bipolar DNA Methylation. Sci Rep. 2016 Sep 2;6(1):32298.
9. Richetto J, Meyer U. Epigenetic Modifications in Schizophrenia and Related Disorders: Molecular Scars of Environmental Exposures and Source of Phenotypic Variability. Biological Psychiatry. 2021 Feb 1;89(3):215–26.
10. Merikangas AK, Shelly M, Knighton A, Kotler N, Tanenbaum N, Almasy L. What genes are differentially expressed in individuals with schizophrenia? A systematic review. Mol Psychiatry. 2022 Mar;27(3):1373–83.
11. Collado-Torres L, Burke EE, Peterson A, Shin J, Straub RE, Rajpurohit A, et al. Regional Heterogeneity in Gene Expression, Regulation, and Coherence in the Frontal Cortex and Hippocampus across Development and Schizophrenia. Neuron. 2019 Jul 17;103(2):203-216.e8.
12. Huang HS, Akbarian S. GAD1 mRNA Expression and DNA Methylation in Prefrontal Cortex of Subjects with Schizophrenia. PLOS ONE. 2007 Aug 29;2(8):e809.
13. Iwamoto K, Bundo M, Yamada K, Takao H, Iwayama-Shigeno Y, Yoshikawa T, et al. DNA Methylation Status of SOX10 Correlates with Its Downregulation and Oligodendrocyte Dysfunction in Schizophrenia. J Neurosci. 2005 Jun 1;25(22):5376–81.
14. Kordi-Tamandani DM, Sahranavard R, Torkamanzehi A. Analysis of association between dopamine receptor genes' methylation and their expression profile with the risk of schizophrenia. Psychiatric Genetics. 2013 Oct;23(5):183.
15. Kundakovic M, Chen Y, Costa E, Grayson DR. DNA Methyltransferase Inhibitors Coordinately Induce Expression of the Human Reelin and Glutamic Acid Decarboxylase 67 Genes. Mol Pharmacol. 2007 Mar 1;71(3):644–53.
16. Putz U, Harwell C, Nedivi E. Soluble CPG15 expressed during early development rescues cortical progenitors from apoptosis. Nat Neurosci. 2005 Mar;8(3):322–31.

17. Javaherian A, Cline HT. Coordinated motor neuron axon growth and neuromuscular synaptogenesis are promoted by CPG15 in vivo. Neuron. 2005 Feb 17;45(4):505–12.

18. Flavell SW, Greenberg ME. Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. Annu Rev Neurosci. 2008;31:563–90.

19. Loebrich S, Nedivi E. The function of activity-regulated genes in the nervous system. Physiol Rev. 2009 Oct;89(4):1079–103.

20. Hallmayer JF, Kalaydjieva L, Badcock J, Dragović M, Howell S, Michie PT, et al. Genetic Evidence for a Distinct Subtype of Schizophrenia Characterized by Pervasive Cognitive Deficit. The American Journal of Human Genetics. 2005 Sep 1;77(3):468–76.

21. Almodóvar-Payá C, Guardiola-Ripoll M, Giralt-López M, Gallego C, Salgado-Pineda P, Miret S, et al. NRN1 Gene as a Potential Marker of Early-Onset Schizophrenia: Evidence from Genetic and Neuroimaging Approaches. International Journal of Molecular Sciences. 2022 Jan;23(13):7456.

22. Almodóvar-Payá C, Guardiola-Ripoll M, Giralt-López M, Oscoz-Irurozqui M, Canales-Rodríguez EJ, Madre M, et al. NRN1 epistasis with BDNF and CACNA1C: mediation effects on symptom severity through neuroanatomical changes in schizophrenia. Brain Struct Funct. 2024 Jun 1;229(5):1299–315.

23. Fatjó-Vilas M, Prats C, Pomarol-Clotet E, Lázaro L, Moreno C, González-Ortega I, et al. Involvement of NRN1 gene in schizophrenia-spectrum and bipolar disorders and its impact on age at onset and cognitive functioning. The World Journal of Biological Psychiatry. 2016 Feb 17;17(2):129–39.

24. Chandler D, Dragović M, Cooper M, Badcock JC, Mullin BH, Faulkner D, et al. Impact of Neuritin 1 (NRN1) polymorphisms on fluid intelligence in schizophrenia. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2010;153B(2):428–37.

25. Bowen EFW, Burgess JL, Granger R, Kleinman JE, Rhodes CH. DLPFC transcriptome defines two molecular subtypes of schizophrenia. Transl Psychiatry. 2019 May 9;9(1):1–10.

26. Pidsley R, Viana J, Hannon E, Spiers H, Troakes C, Al-Saraj S, et al. Methylomic profiling of human brain tissue supports a neurodevelopmental origin for schizophrenia. Genome Biol. 2014;15(10):483.

27. Reshetnikov VV, Kisaretova PE, Ershov NI, Shulyupova AS, Oshchepkov DY, Klimova NV, et al. Genes associated with cognitive performance in the Morris water maze: an RNA-seq study. Sci Rep. 2020 Dec 16;10(1):22078.

28. Wan K, Mao F, Li Q, Wang L, Wei Z, Wang P, et al. Neuritin-overexpressing transgenic mice demonstrate enhanced neuroregeneration capacity and improved spatial learning and memory recovery after ischemia-reperfusion injury. Aging (Albany NY). 2020 Dec 15;13(2):2681–99.

29. Liu Y ping, Wu X, Xia X, Yao J, Wang B jie. The genome-wide supported CACNA1C gene polymorphisms and the risk of schizophrenia: an updated meta-analysis. BMC Medical Genetics. 2020 Aug 8;21(1):159.

30. Alme MN, Wibrand K, Dagestad G, Bramham CR. Chronic Fluoxetine Treatment Induces Brain Region-Specific Upregulation of Genes Associated with BDNF-Induced Long-Term Potentiation. Neural Plast. 2007;2007:26496.

31. Park HG, Yu HS, Park S, Ahn YM, Kim YS, Kim SH. Repeated treatment with electroconvulsive seizures induces HDAC2 expression and down-regulation of NMDA receptor-related genes through histone deacetylation in the rat frontal cortex. International Journal of Neuropsychopharmacology. 2014 Sep 1;17(9):1487–500.

32. Lally J, Ajnakina O, Forti MD, Trotta A, Demjaha A, Kolliakou A, et al. Two distinct patterns of treatment resistance: clinical predictors of treatment resistance in first-episode schizophrenia spectrum psychoses. Psychological Medicine. 2016 Nov;46(15):3231–40.

33. Samara MT, Dold M, Gianatsi M, Nikolakopoulou A, Helfer B, Salanti G, et al. Efficacy, Acceptability, and Tolerability of Antipsychotics in Treatment-Resistant Schizophrenia: A Network Meta-analysis. JAMA Psychiatry. 2016 Mar 1;73(3):199–210.

34. de Bartolomeis A, Vellucci L, Barone A, Manchia M, De Luca V, Iasevoli F, et al. Clozapine's multiple cellular mechanisms: What do we know after more than fifty years? A systematic review and critical assessment of translational mechanisms relevant for innovative strategies in treatment-resistant schizophrenia. Pharmacology & Therapeutics. 2022 Aug 1;236:108236.

35. Guidotti A, Dong E, Grayson DR. Epigenetic Basis of Clozapine Action. 2017;

36. Lee BJ, Marchionni L, Andrews CE, Norris AL, Nucifora LG, Wu YC, et al. Analysis of differential gene expression mediated by clozapine in human postmortem brains. Schizophrenia Research. 2017 Jul;185:58–66.

37. Prats C, Arias B, Ortet G, Ibáñez MI, Moya J, Pomarol-Clotet E, et al. Role of neurotrophins in depressive symptoms and executive function: Association analysis of NRN1 gene and its interaction with BDNF gene in a non-clinical sample. Journal of Affective Disorders. 2017 Mar 15;211:92–8.

38. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. Am J Hum Genet. 2007 Sep;81(3):559–75.

39. Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods. 2001 Dec 1;25(4):402–8.

40. Redlich R, Schneider I, Kerkenberg N, Opel N, Bauhaus J, Enneking V, et al. The role of BDNF methylation and Val66Met in amygdala reactivity during emotion processing. Human Brain Mapping. 2020;41(3):594–604.

41. Thompson M, Hill BL, Rakocz N, Chiang JN, Geschwind D, Sankararaman S, et al. Methylation risk scores are associated with a collection of phenotypes within electronic health record systems. npj Genom Med. 2022 Aug 25;7(1):1–11.

42. Rohart F, Gautier B, Singh A, Cao KAL. mixOmics: An R package for 'omics feature selection and multiple data integration. PLOS Computational Biology. 2017 Nov 3;13(11):e1005752.

43. Morgan SE, Seidlitz J, Whitaker KJ, Romero-Garcia R, Clifton NE, Scarpazza C, et al. Cortical patterning of abnormal morphometric similarity in psychosis is associated with brain expression of schizophrenia-related genes. Proceedings of the National Academy of Sciences. 2019 May 7;116(19):9604–9.

44. Fernandez-Egea E, Vértes PE, Flint SM, Turner L, Mustafa S, Hatton A, et al. Peripheral Immune Cell Populations Associated with Cognitive Deficits and Negative Symptoms of Treatment-Resistant Schizophrenia. PLOS ONE. 2016 May 31;11(5):e0155631.

45. Rizig MA, McQuillin A, Ng A, Robinson M, Harrison A, Zvelebil M, et al. A gene expression and systems pathway analysis of the effects of clozapine compared to haloperidol in the mouse brain implicates susceptibility genes for schizophrenia. J Psychopharmacol. 2012 Sep 1;26(9):1218–30.

46. Lu JM, Liu DD, Li ZY, Ling C, Mei YA. Neuritin Enhances Synaptic Transmission in Medial Prefrontal Cortex in Mice by Increasing CaV3.3 Surface Expression. Cerebral Cortex. 2017 Jul 1;27(7):3842–55.

47. Yao JJ, Gao XF, Chow CW, Zhan XQ, Hu CL, Mei YA. Neuritin Activates Insulin Receptor Pathway to Up-regulate Kv4.2-mediated Transient Outward K+ Current in Rat Cerebellar Granule Neurons *. Journal of Biological Chemistry. 2012 Nov 30;287(49):41534–45.

48. Zhao QR, Lu JM, Li ZY, Mei YA. Neuritin promotes neurite and spine growth in rat cerebellar granule cells via L-type calcium channel-mediated calcium influx. Journal of Neurochemistry. 2018;147(1):40–57.

49. Ibi D, de la Fuente Revenga M, Kezunovic N, Muguruza C, Saunders JM, Gaitonde SA, et al. Antipsychotic-induced Hdac2 transcription via NF-κB leads to synaptic and cognitive side effects. Nat Neurosci. 2017 Sep;20(9):1247–59.

50. Dong E, Nelson M, Grayson DR, Costa E, Guidotti A. Clozapine and sulpiride but not haloperidol or olanzapine activate brain DNA demethylation. Proceedings of the National Academy of Sciences of the United States of America. 2008 Aug 29;105(36):13614.

51. Alelú-Paz R, Carmona FJ, Sanchez-Mut JV, Cariaga-Martínez A, González-Corpas A, Ashour N, et al. Epigenetics in Schizophrenia: A Pilot Study of Global DNA Methylation in Different Brain Regions Associated with Higher Cognitive Functions. Front Psychol [Internet]. 2016 Sep 30 [cited 2024 Jan 8];7. Available from: http://journal.frontiersin.org/Article/10.3389/fpsyg.2016.01496/abstract

52. Farsi Z, Nicolella A, Simmons SK, Aryal S, Shepard N, Brenner K, et al. Brain-region-specific changes in neurons and glia and dysregulation of dopamine signaling in Grin2a mutant mice. Neuron. 2023 Nov 1;111(21):3378-3396.e9.

53. Huckins LM, Dobbyn A, Ruderfer DM, Hoffman G, Wang W, Pardiñas AF, et al. Gene expression imputation across multiple brain regions provides insights into schizophrenia risk. Nat Genet. 2019 Apr;51(4):659–74.

54. Chen C, Zhang C, Cheng L, Reilly JL, Bishop JR, Sweeney JA, et al. Correlation between DNA methylation and gene expression in the brains of patients with bipolar disorder and schizophrenia. Bipolar Disorders. 2014;16(8):790–9.

55. Dong E, Tueting P, Matrisciano F, Grayson DR, Guidotti A. Behavioral and molecular neuroepigenetic alterations in prenatally stressed mice: relevance for the study of chromatin remodeling properties of antipsychotic drugs. Transl Psychiatry. 2016 Jan;6(1):e711–e711.

56. Dunham I, Kundaje A, Aldred SF, Collins PJ, Davis CA, Doyle F, et al. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012 Sep;489(7414):57–74.

57. Griffith OL, Montgomery SB, Bernier B, Chu B, Kasaian K, Aerts S, et al. ORegAnno: an open-access community-driven resource for regulatory annotation. Nucleic Acids Res. 2008 Jan;36(Database issue):D107–13.

58. Kyosseva SV, Elbein AD, Hutton TL, Griffin WST, Mrak RE, Sturner WQ, et al. Increased Levels of Transcription Factors Elk-1, Cyclic Adenosine Monophosphate Response Element-Binding Protein, and Activating Transcription Factor 2 in the Cerebellar Vermis of Schizophrenic Patients. Archives of General Psychiatry. 2000 Jul 1;57(7):685–91.

59. Kriegebaum CB, Gutknecht L, Bartke L, Reif A, Buttenschon HN, Mors O, et al. The expression of the transcription factor FEV in adult human brain and its association with affective disorders. J Neural Transm. 2010 Jul 1;117(7):831–6.

60. Tay N, Macare C, Liu Y, Ruggeri B, Jia T, Chu C, et al. Allele-Specific Methylation of SPDEF: A Novel Moderator of Psychosocial Stress and Substance Abuse. AJP. 2019 Feb;176(2):146–55.

61. Duclot F, Kabbaj M. The Role of Early Growth Response 1 (EGR1) in Brain Plasticity and Neuropsychiatric Disorders. Front Behav Neurosci [Internet]. 2017 Mar 6 [cited 2024 Jul 26];11. Available from: https://www.frontiersin.org/journals/behavioral-neuroscience/articles/10.3389/fnbeh.2017.00035/full

62. Gaunt TR, Shihab HA, Hemani G, Min JL, Woodward G, Lyttleton O, et al. Systematic identification of genetic influences on methylation across the human life course. Genome Biol. 2016 Mar 31;17(1):61.

63. Ward LD, Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. Nucleic Acids Res. 2016 Jan 4;44(Database issue):D877–81.

Section III: The impact of *NRN1* genetic variability and peripheral epigenetic changes on cognitive improvements following Cognitive Remediation Therapy (CRT)

Study III

NRN1 genetic variability and methylation changes as biomarkers for cognitive remediation therapy response in schizophrenia

Almodóvar-Payá C, París-Gómez I, Latorre-Guardia M, Guardiola-Ripoll M, Catalán R, Arias B, Gallego C, Penadés R, Fatjó-Vilas M

Progress in Neuropsychopharmacology & Biological Psychiatry. 2024 Oct 18;136:111175..

doi: 10.1016/j.pnpbp.2024.111175. Epub ahead of print. PMID: 39426559

Progress in Neuropsychopharmacology & Biological Psychiatry 136 (2025) 111175

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

NRN1 genetic variability and methylation changes as biomarkers for cognitive remediation therapy response in schizophrenia

Carmen Almodóvar-Payá [a,b,c], Irene París-Gómez [a], Mariona Latorre-Guardia [a,b], Maria Guardiola-Ripoll [d], Rosa Catalán [b,e,g,i], Bárbara Arias [b,c,f], Rafael Penadés [b,g,h,i,1,*], Mar Fatjó-Vilas [a,b,c,*,1]

[a] FIDMAG Germanes Hospitalàries Research Foundation, Barcelona, Spain
[b] Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Spain
[c] Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain
[d] Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Spain
[e] Departament de Medicina, Campus Clínic, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona, Barcelona, Spain
[f] Institut de Biomedicina de la Universitat de Barcelona (IBUB), Barcelona, Spain
[g] Barcelona Clinic Schizophrenia Unit (BCSU), Hospital Clínic, Barcelona, Spain
[h] Departament de Psicologia Clínica i Psicobiologia, Facultat de Psicologia, Universitat de Barcelona, Barcelona, Spain
[i] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

ARTICLE INFO

Keywords:
Schizophrenia
Cognition
CRT
Methylation
SNP
NRN1

ABSTRACT

Cognitive remediation therapy (CRT) demonstrates potential in enhancing cognitive function in schizophrenia (SZ), though the identification of molecular biomarkers remains challenging. The Neuritin-1 gene (*NRN1*) emerges as a promising candidate gene due to its association with SZ, cognitive performance and response to neurotherapeutic treatments. We aimed to investigate whether *NRN1* genetic variability and methylation changes following CRT are related to cognitive improvements.

Twenty-five SZ patients were randomly assigned to CRT or treatment-as-usual (TAU) groups, with cognitive function and *NRN1* methylation assessed pre- and post-intervention using the MATRICS Consensus Cognitive Battery and EpiTYPER. Besides, eleven *NRN1* polymorphisms were genotyped. Methylation changes (Δm = post - pre) were analyzed via sparse Partial Least Square Discriminant Analysis (sPLS-DA) to identify latent components (LCs) distinguishing CRT from TAU. To further explore methylation patterns of these LCs, CpG units were grouped into two subsets, yielding Δm means for those with increased and decreased methylation. Cognitive changes (Δcog = post - pre) were used to identify CRT improvers (CRT-I, Δcog ≥ 1), and the association between methylation changes and cognitive improvements post-therapy was also tested.

We identified two LCs that differentiated CRT from TAU with a classification error rate of 0.28. The main component, LC1, included 25 CpG units. The subsets of CpG units with increased and decreased post-therapy methylation differed significantly between the two treatment arms, suggesting that differences were not merely data-driven but reflected meaningful biological variation. Additionally, CpG units linked to therapy were also associated with cognitive improvement, with LC1 and the subset of CpG units showing increased methylation post-therapy distinguishing CRT-I from the rest of the patients across multiple cognitive domains. Furthermore, the effect of LC1 on speed processing improvement after CRT was enhanced by considering the *NRN1*-rs9405890 polymorphism. Notably, these CpG units, particularly those with increased methylation after CRT, overlapped with key gene regulatory elements.

Our model, integrating genetics and epigenetics, boosts the understanding of CRT response variability and highlights this multi-level approach as a promising strategy for identifying potential *NRN1*-related biomarkers of CRT effects, though further studies with larger samples are needed.

* Corresponding authors at: Barcelona Clinic Schizophrenia Unit, Hospital Clínic, Barcelona, Spain (R Penadés) and FIDMAG Germanes Hospitalàries Research Foundation, Barcelona, Spain (M Fatjó-Vilas). and Barcelona Clinic Schizophrenia Unit, Hospital Clínic, Barcelona, Spain (R Penadés).
E mail addresses: rpenades@clinic.cat (R. Penadés), mfatjo-vilas@fidmag.org (M. Fatjó-Vilas).
[1] Co-senior authors: Mar Fatjó-Vilas and Rafael Penadès

https://doi.org/10.1016/j.pnpbp.2024.111175
Received 11 June 2024; Received in revised form 20 September 2024; Accepted 15 October 2024
Available online 18 October 2024

1. Background

Schizophrenia (SZ) is a complex brain disorder, impacting approximately 0.3–0.7 % of the global population (Charlson et al., 2018) and emerging from a combination of genetic and environmental factors influencing neurodevelopmental trajectories (Schmitt et al., 2023). With a significant genetic component, indicated by an 80 % heritability and numerous common genetic variants associated (Hilker et al., 2018; Sullivan et al., 2003; Trubetskoy et al., 2022), the high 50 % discordance among monozygotic twins and the modest 24 % of SZ's liability explained by common genetic variants reveal the influence of non-genetic and environmental influences (Petronis et al., 2003; Trubetskoy et al., 2022).

This fact has prompted research in epigenetics, dynamic processes that influence gene expression by altering the chromatin state (Khavari and Cairns, 2020). Prominently studied among these mechanisms is DNA methylation, characterized by the addition of methyl groups to the cytosine bases of the DNA molecule, particularly those followed by guanine and referred to as CpG sites that often group in specific genomic regions known as CpG islands (Moore et al., 2013; Schübeler, 2015). However, the relationship between methylation and gene expression is believed to be complex, with the balance between methylation levels in the promoter and gene body regions being crucial for effective regulation (Wagner et al., 2014). It is well-described that methylation processes are influenced by environmental stimuli (Jirtle and Skinner, 2007), leading to changes, some of which may have lasting effects, thus directly impacting gene expression and shaping the phenotype (Jaenisch and Bird, 2003; Perera et al., 2020). Consequently, the extent of methylation at specific CpG sites can be attributed partially to the DNA sequence, environmental factors, or a combination of both (Shah et al., 2014).

Several peripheral methylome-wide association studies have identified methylation variances between SZ patients and healthy controls (Aberg et al., 2014; Hannon et al., 2016; Montano et al., 2016), but also between monozygotic twins discordant for the disorder (Dempster et al., 2011; Fisher et al., 2015). Interestingly, a meta-analysis has demonstrated that numerous studies converge in detecting a significant cluster of genes associated with synaptic membrane function and structure, alongside others involved in immune and stress responses (Chan et al., 2020). Additionally, some studies have indicated that peripheral methylation changes are also present in ultra-high-risk individuals (Ciuculete et al., 2017) and even that specific methylomic changes appear during the conversion to psychosis (Kebir et al., 2017).

Although peripheral methylation may not capture all brain-related disease changes, some studies have shown promise by revealing significant correlations in DNA methylation between blood and brain tissue (Davies et al., 2012; Walton et al., 2016). Notwithstanding, beyond these tissue correlations, investigating blood methylation remains valuable for biomarker identification, particularly given the accessibility of the tissue and its reversible nature, which enables the examination of therapy-induced changes potentially leading to enhanced patient care (Goud Alladi et al., 2018). In this sense, exploring methylation's connection to cognition in SZ is particularly compelling, as cognitive symptoms constitute a core dimension significantly affected in SZ, but also because it remains inadequately addressed by existing pharmacological treatments (Nielsen et al., 2015). These cognitive symptoms encompass declines in memory, language, executive functions, processing speed, and deficits in social cognition, including impaired theory of mind (Fatouros-Bergman et al., 2014). Moreover, they can manifest in ultra-high-risk individuals (Bora et al., 2014) and, to a milder extent, in unaffected relatives (Bora, 2017). Importantly, these symptoms may precede and persist beyond positive (e.g., delusions, hallucinations) and negative symptoms (e.g., lack of volition), influencing functionality and contributing to disability (Jonas et al., 2022). Thus, it has been suggested that the most effective strategy for enhancing or normalizing cognition in SZ has to involve a combination of pharmacological medication and psychological therapies (Michalopoulou et al., 2013).

In this scenario, cognitive remediation therapy (CRT), a learning-based intervention, has shown promising results in alleviating global cognitive deficits in SZ (Wykes et al., 2002). Anchored in the principle of neuroplasticity, neuroimaging studies consistently reveal increased bilateral activation in prefrontal and parietal areas as the most common finding associated with CRT (Mothersill and Donohoe, 2019; Penadés et al., 2017). Furthermore, changes in neuronal-connectivity (Penadés et al., 2020a, 2020b; Ramsay et al., 2017), along with structural modifications in white matter (Matsuoka et al., 2019; Penadés et al., 2013) and grey matter (Eack et al., 2010; Morimoto et al., 2018), have been observed. Interestingly, these functional and structural changes correlated with behavioral improvements in most of the mentioned studies. However, despite these advances, little is known about the molecular basis underlying the effectiveness of CRT, even though it has been suggested that epigenetic mechanisms may play a pivotal role in understanding the biological insights associated with various types of psychotherapy (Penadés et al., 2020a, 2020b).

Among the genes essential in neurodevelopment and intimately involved in synaptic plasticity there is Neuritin-1 (*NRN1)*, located at chromosome 6p25.1 and initially identified in a screening for activity-regulated genes by Nedivi et al., 1996. In first stages of development, *NRN1* acts as a survival factor for neuronal progenitors and differentiated neurons (Putz et al., 2005). In later stages, it also facilitates the growth and stabilization of axonal and dendritic arbors and the formation and maturation of synapses (Javaherian and Cline, 2005). In the adult brain, *NRN1* expression is correlated with activity-dependent functional synaptic plasticity, particularly in the hippocampus and neocortex (Flavell and Greenberg, 2008; Loebrich and Nedivi, 2009). Regarding its role in cognitive processes, linkage studies have related *NRN1* chromosomal region to intelligence quotient (IQ) (Posthuma et al., 2005), and genetic association studies have identified diverse common variants to influence executive function (Prats et al., 2017). In the context of SZ, the *NRN1* chromosomal region has been linked to a subtype marked by cognitive deficits (Hallmayer et al., 2005), and genetic association studies have also implicated *NRN1* variability in SZ risk, cognitive deficits, early onset, as well as brain structural and functional changes (Almodóvar-Payá et al., 2022, 2024; Chandler et al., 2010; Fatjó-Vilas et al., 2016). Additionally, a methylome-wide association study has identified *NRN1* as one of the most differentially methylated genes in the cortex of individuals with SZ (Pidsley et al., 2014). Parallelly, beyond studies in SZ, *NRN1* brain expression has been linked to cognitive resilience against Alzheimer's disease (Hurst et al., 2023; Johnson et al., 2022; Yu et al., 2020).

However, despite the crucial roles of *NRN1* in synaptic plasticity and cognition, no previous study has explored the role of *NRN1* as a biomarker of CRT response. Accordingly, in the current study we hypothesized that *NRN1* genetic variability and methylation changes after CRT will be associated with therapy-derived cognitive improvement. This hypothesis was tested in a two-arm randomized controlled trial, with participants randomly assigned to receive either CRT or treatment as usual (TAU), and *NRN1* methylation levels and cognitive function assessed before and after the intervention.

2. Methods

2.1. Design

This study builds on Penadés et al., 2024 research, a two-arm randomized controlled trial (registered as NCT04278027). Participants were randomized to receive either cognitive remediation therapy (CRT or therapy group) or treatment as usual (TAU or control group) with a 2:1 ratio. Randomization was carried out by a psychiatrist who was not involved in the assignment process and was solely responsible for generating the allocation sequence. The sequence was created using a free, web-based program (www.randomizer.org). To ensure blinding and prevent bias, sequentially numbered, opaque sealed envelopes with carbon paper, as detailed in the SNOSE procedure (Doig and Simpson, 2005), were used for the assignments. Outcome and explanatory variables were assessed before (pre = baseline) and after treatment (post = 16 weeks) blind to group assignment.

Participants were recruited by the staff of Barcelona Clinic Schizophrenia Unit (BCSU) at the Hospital Clinic of Barcelona. Sixty patients with SZ, classified according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), were assessed for eligibility based on the criteria determined by the Structured Clinical Interview for DSM-5 (SCID-5) by First et al., 2002. All met the following inclusion criteria: i) diagnosis of SZ according to DSM-5 confirmed by SCID, ii) stability of symptoms, verified by a psychiatrist, ensuring participants had been on a stable dose of antipsychotic medication and had no hospitalizations or acute episodes for at least six months before the trial, and iii) ongoing clozapine treatment, indicative of poorer functional outcomes (Iasevoli et al., 2016), with no planned modifications in antipsychotic medication. From the initial assessment, some participants were excluded based on the exclusion criteria: i) presence of neurological or traumatic brain conditions, ii) changes in antipsychotic dosage greater than 10 % between pre- and post-intervention, and iii) abuse of psychotropic substances. Then, the final sample included in the trial was composed of 60 participants (40 CRT and 20 TAU). After excluding those without biological samples available both at pre- and post-therapy, the samples available for the methylation analyses were 43.

Symptoms evaluation was performed using the Spanish version of the Positive and Negative Syndrome Scale (PANSS) at baseline (Kay et al., 1987), which can be divided into three subscales for measuring the severity of general psychopathology, positive symptoms, and negative symptoms.

2.2. Interventions

Regardless of group assignment, all participants from both treatment arms continued to receive standard care, including their usual pharmacological and psychological treatments as needed, and underwent four monthly visits with clinical psychiatrists to monitor their psychopathological course and aspects related to pharmacological treatment, following the protocol of the clinical unit where patients were attended.

2.2.1. Cognitive remediation therapy (CRT)

The participants in the therapy group underwent a Cognitive Remediation Therapy (CRT) protocol using the CIRCuiTS software (Computerized Interactive Remediation of Cognition and Thinking Skills Reeder and Wykes, 2010). This therapist-supported web-based program facilitated cognitive remediation tasks. Additionally, participants engaged in supplementary and independent sessions aimed at promoting mass practice, enhancing the intensity and frequency of cognitive task practice. The trial spanned two years, during which evaluation and treatment procedures were applied to the participants. The treatment consisted of up to 40 sessions, held 3 times per week over a period of up to 16 weeks (Reeder et al., 2017).

2.2.2. Treatment as usual (TAU)

The control group received only standard antipsychotic medication without any psychological or cognitive treatment during the trial.

2.3. Cognitive evaluation

Research assistants blinded to the group assignments of patients evaluated the cognitive performance using the Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery (MATRICS Consensus Cognitive Battery - MCCB (Kern et al., 2008; Nuechterlein et al., 2008). It is organized into six domains (speed of processing, attention/vigilance, working memory, verbal and visual learning, and reasoning and problem-solving). Following the recommendation of the battery developers, a global composite score was calculated by averaging the t-scores of all tests, which is called global cognition. The first assessment was conducted one week before the therapy started, and the final assessment took place one week after the therapy concluded.

2.4. Sample description

We used a representative subset of 25 individuals from the prior study by Penadés et al. (2024) to conduct the present study. First, we randomly selected the 16 CRT participants, and then chose the 9 TAU participants, ensuring they were matched by sex and age. Additionally, we verified that there were no differences between the two treatment arms in any other demographic characteristics or clinical profile, including evaluation of symptom severity with the PANSS (Table 1). Furthermore, to confirm that this subsample was equivalent to the initial one, we checked that there were no significant differences in any baseline cognitive, demographic, or clinical variables (**Supplementary**

Table 1
Sample characteristics, encompassing both demographic and clinical details, of subjects undergoing cognitive remediation therapy (CRT) or receiving treatment as usual (TAU). Demographically, the table provides sex distribution, including the count and percentage (%) of males, alongside age and education level reported in years. Clinically, the table includes the age of onset and illness duration expressed in years, as well as hospitalizations representing the number of inpatient stays and antipsychotic dosage specified as clozapine mg/day. Also, the scores on the Positive and Negative Symptoms Scale (PANSS) are given. Quantitative variables are accompanied by their respective mean and standard deviation (SD).

	CRT ($n = 16$)	TAU ($n = 9$)	χ^2 / t	p-value
Sex (M/F)	10/6 (62.5 %)	6/3 (66.6 %)	0.04	0.84
Age	40 (8.20)	42 (7.02)	−0.89	0.38
Education	11.25 (3.29)	11.33 (3.71)	−0.58	0.95
Age at onset	24.81 (8.36)	21.67 (3.61)	1.07	0.29
Illness duration	15.69 (7.76)	21.44 (6.98)	−0.84	0.08
Number of hospitalizations	2.31 (1.14)	2.27 (1.50)	−0.67	0.51
Antipsychotic dosage	329.69 (175.17)	319.44 (152.98)	0.15	0.88
PANSS Total	82.75 (18.34)	88.22 (11.80)	0.08	0.94
PANSS Positive	12.88 (4.80)	15.00 (6.02)	−0.97	0.34
PANSS Negative	23.63 (6.53)	21.33 (4.24)	0.94	0.36
PANSS General	46.25 (10.14)	45.59 (7.08)	0.09	0.93

Table S1).

2.5. Molecular variables

For all individuals, genomic DNA was obtained from peripheral blood cells at baseline (pre) and after treatment (post), and extracted using Realpure SSS Kit for DNA Extraction (Durviz, S.L.U, Valencia, Spain). This extracted DNA was employed in genotyping and methylation analyses.

2.5.1. NRN1 methylation

Methylation analyses targeted the CpGs along the three CpG islands (CGI) spanning the *NRN1* gene (CGI1 chr6:5,998,917–5,999,730, CGI2 chr6:6,002,288–6,004,772 and CGI3 chr6:6,006,457–6,006,810, reference genome UCSC-hg38). Samples were analyzed using the EpiTYPER method (Agena Bioscience) at the Centro Nacional de Genotipado (CEGEN, http://usc.es/cegen/). This method examines CpG sites within amplicons ranging from 200 to 600 base pairs, being especially well-suited for endeavors aimed at investigating specific regions. In our case, six amplicons were designed using Agena's EpiDesigner software to amplify these regions of interest (**Supplementary Table S2**).

The methylation values can range between 0 and 1, being 0 the value corresponding to a 0 % methylation state and 1 the value associated with a 100 % methylated state. However, some CpG sites are located too close to independently determine their methylation levels; therefore, the methylation status of a fragment containing multiple CpG sites is assessed. In this case, the methylation status of a fragment reflects the mean of all CpG sites within that fragment. Therefore, we will refer to CpG units, which can comprise one or more CpG sites. Each CpG unit was denoted numerically based on its 5′ to 3′ genomic position on the forward strand sequence. The methylation quality control protocol implemented by CEGEN involved excluding units deviating by over 10 % from established commercial controls (HMc, 100 % methylated, and LMc, 0 % methylated), as well as excluding samples with standard deviation variances between replicas exceeding 10 %. In all, we have analyzed 63 CpG units (consisting of 182 CpG sites) along 3 CGIs within the *NRN1* region (Fig. 1 and **Supplementary Table S3**).

2.5.2. NRN1 genotyping

We genotyped 11 Single Nucleotide Polymorphisms (SNP) at the Neuritin1 gene (*NRN1*, 6p25.1). The genotyping was carried out at the National Genotyping Center (CeGen, http://usc.es/cegen/). The 11

Fig. 1. Schematic representation of the *NRN1* gene (NCBI36/hg18) with exonic (in black) and CpG island (CGI, in blue) regions, as well as single nucleotide polymorphisms (SNPs) included in the study placed according to the human genome browser (http://genome.ucsc.edu). Positions of the analyzed CpG sites in the DNA sequence are indicated below (in bold blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Information regarding *NRN1* Single Nucleotide Polymorphisms (SNPs) included in this study. The table contains the dbSNP number, chromosome location, alleles (minor/major), and minor allele frequency (MAF) for each SNP based on the UCSC Genome Browser on Human (GRCh38/hg38). Additionally, the table provides MAF and genotypic count and frequency (%) separately for subjects undergoing cognitive remediation therapy (CRT) and treatment as usual (TAU). Due to low frequencies in some genotypes, frequencies shown in the last two columns and subsequent analyses were conducted with all SNPs dichotomized (homozygous and heterozygous for the minor allele / homozygous for the major allele).

SNPs	Chr Position	Allele	MAF_{1000G}	MAF_{sample}	CRT	TAU
rs2208870	6:5992257	G/A	0.34	0.34	10 (62.5 %) / 6	4 (44.4 %) /4
rs12333117	6:5994759	T/C	0.40	0.31	10 (62.5 %) / 6	7 (77.75) / 2
rs582186	6:6001148	A/G	0.38	0.40	11 (73.3 %) / 5	6 (66.6 %) / 3
rs645649	6:6004726	C/G	0.36	0.34	10 (62.5 %) / 6	4 (44.4 %) / 5
rs582262	6:6007758	C/G	0.30	0.31	10 (62.5 %) / 6	3 (33.3 %) / 5
rs3763180	6:6009615	T/G	0.46	0.41	12 (75 %) / 4	3 (33.3 %) / 5
rs10484320	6:6010204	T/C	0.22	0.18	6 (62.5 %) / 10	3 (33.3 %) / 5
rs4960155	6:6010306	C/T	0.49	0.41	12 (75 %) / 4	6 (66.6 %) / 3
rs9379002	6:6012158	G/T	0.29	0.41	11(73.3 %) / 5	4 (44.4 %) / 4
rs9405890	6:6012488	C/T	0.31	0.37	11 (73.3 %) / 5	4 (44.4 %) / 5
rs1475157	6:6016936	G/A	0.17	0.18	6 (62.5 %) / 10	2 (22.2 %) / 7

SNPs included in the study were chosen to cover the genomic sequence and approximately 10 kb upstream and downstream. The optimal set of SNPs, which contained the maximum information about surrounding variants, was selected using the SYSNPs tool (http://www.sysnps.org/) with a minor allele frequency (MAF) > 5 %, utilizing the pairwise tagging option ($r^2 \geq 0.8$). Additionally, we included SNPs previously associated with SZ in the study by Chandler et al. (2010). All these SNPs have also been genotyped in other studies conducted by our group (Almodóvar-Payá et al., 2022, 2024; Fatjó-Vilas et al., 2016; Prats et al., 2017). As shown in Table 2, the genotyping call rate was 99 % and the minor allele frequencies were comparable to the ones described for the EUR population in the 1000 Genomes Project. Furthermore, genotype frequencies were confirmed to be in Hardy-Weinberg equilibrium (PLINK v.1.09).

2.6. Statistical analyses

2.6.1. Characteristics of the sample and CRT benefits in cognition

For mean comparisons between CRT and TAU in relation to socio-demographic data, we used t-student (for quantitative variables) and chi-square (for qualitative variables) tests.

We applied repeated measures ANOVA to determine if the observed changes in cognition between pre and post differed between the treatment groups. The main factor of interest was the interaction of time (pre-post) with treatment (CRT/TAU). A significant interaction means that slopes observed between pre-post measures differ between treatment arms. Age, sex, illness duration, and antipsychotic dosage (clozapine in mg/day) were included as adjusting variables.

2.6.2. Between-groups NRN1 methylation changes over time

Prior to analyzing methylation data, the confounding variables (age, sex, illness duration, and clozapine dosage) were regressed from each predictor methylation variable (CpGs). To get a measure of the methylation change, the baseline methylation residuals were subtracted from the methylation residuals after the treatment (Δm = post - pre).

Due to the high intercorrelations of CpG units and the associated multicollinearity (**Supplementary Table S4**), we used sparse Partial Least Square-Discriminant Analysis (sPLS-DA) to assess the differences in the Δm values between treatment arms. All the analyses were based on the mixOmics protocol as implemented in the specific R package (Rohart et al., 2017). This method is appropriate for high-dimensional datasets where the number of variables exceeds the number of observations, as it projects the predictor (X) and response (Y) variables into a lower-dimensional space by constructing latent components (LCs) that maximize covariance. To address the challenge posed by the small sample size in our study, we applied Leave-One-Out Cross-Validation (LOOCV) at multiple stages of the modeling process to calculate classification error. LOOCV is particularly well-suited to small datasets as it maximizes each data point's use by treating each individual sample as a test set once, while training the model on the remaining data. First, LOOCV was used to determine the optimal number of LCs by evaluating the classification error across different component configurations. To further optimize the model, we applied the LASSO penalty in combination with LOOCV to select the most relevant CpG units. After determining the optimal number of LCs and CpG units, we used LOOCV to evaluate the performance of the final sPLS-DA model. We interpreted each CpG unit within an LC in two main ways: first, through its coefficient or loading value, which indicates its importance in distinguishing between groups, and second, by assessing the contribution of the CpG, which identifies the group where it shows a higher methylation difference (Δm). Additionally, to validate the reproducibility of the CpG signature captured by the LCs, we performed a feature stability analysis, which assessed how frequently each CpG unit was selected across multiple iterations of the model.

Our criterion for prioritizing the most informative LC were: i) to select the first component, which by the intrinsic methodology of sPLS-DA inherently retains the highest covariance between CpGs and treatment arms, ii) to retain only those other components showing statistical differences between treatment arms using the Mann-Whitney *U* test, and iii) to assess the stability of the features within each LC, as our subsequent analyses focused on exploring the methylation patterns of CpG units.

In addition to applying LOOCV throughout the entire model-building process, we later addressed potential overfitting and assessed the robustness of the model built with optimal parameters by randomly splitting the data into training and test sets and computing key performance metrics using a bootstrapping approach with 500 iterations. The metrics include precision, which measures the proportion of correctly predicted positive instances; recall, which indicates the proportion of actual positives correctly identified; the F1 score, which is the harmonic mean of precision and recall; and accuracy, which represents the overall proportion of correct predictions.

To further explore the methylation patterns of the CpG units included in the LCs and following other studies with similar approaches (Morgan et al., 2019; Fernandez-Egea et al., 2016), we computed two Δm means: one grouping CpG units showing decreased methylation levels from pre to post in CRT group (Δm CRT < Δm TAU, referred to as the "negative change average"); and another grouping CpG units showing increased methylation levels from pre to post in CRT group (Δm CRT > Δm TAU, referred to as the "positive change average"). To validate the robustness of these patterns and ensure that the observed differences were not merely the result of a data-driven process, we also utilized the Mann-Whitney *U* test to statistically compare these means between the two treatment arms.

2.6.3. NRN1 methylation changes associated with CRT cognitive performance improvement

To deepen our understanding of the *NRN1* methylation signature associated with CRT cognitive improvement and to get a measure of the cognitive change, the baseline cognitive scores were subtracted from the cognitive scores after the treatment (Δcog = post - pre). We utilized these values to classify participants into two groups: those who underwent CRT and exhibited cognitive improvement (Δcog ≥1, CRT-I), and those who either did not show improvement following CRT (Δcog <1, CRT-NI) or received TAU (**Supplementary Table S5**). This classification was conducted for each cognitive domain and global cognition. This cut-off for classification was based on recent meta-analyses of CRT effectiveness, which emphasize that although cognitive improvements following CRT are typically modest, they can still result in meaningful clinical benefits and enhanced psychosocial functioning (Vita et al., 2021). Then, we used the Mann-Whitney *U* test to assess the ability of

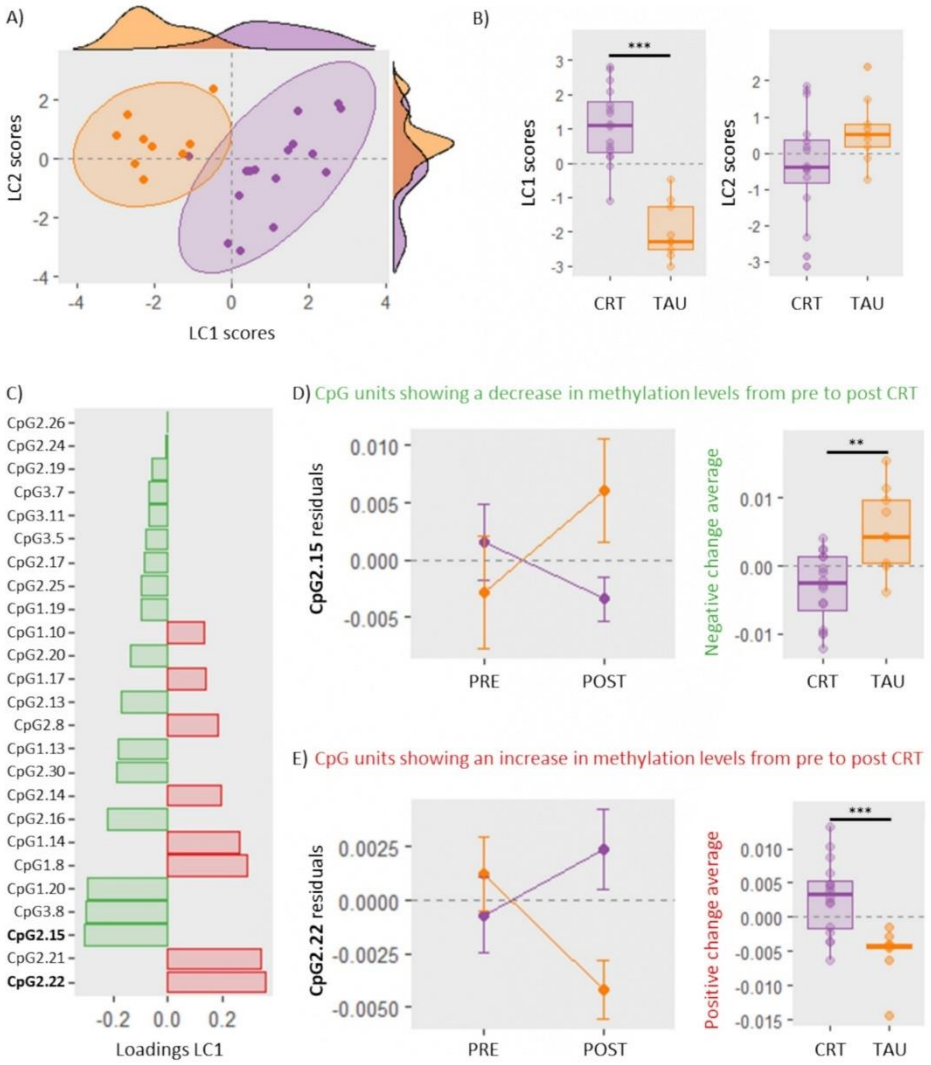


Fig. 2. ***A)*** *Scatter plot projecting samples onto a space defined by the first two latent component (LC) scores from sPLS-DA analysis, with each treatment group, cognitive remediation therapy (CRT) or treatment as usual (TAU), represented by a 95 % confidence ellipse, colored in purple and orange,* respectively. **B)** Box plots displaying LC1 and LC2 scores with ±2 standard errors (SE) shown by the treatment group (CRT and TAU). **C)** Bar plot illustrating loading weights for each CpG unit in LC1, ranked by value. Red bars denote CpG units with higher methylation delta values (Δm = post - pre) in the CRT group, while green bars indicate those with lower Δm. **D and E)** Left: Line plots displaying methylation changes from pre to post assessment for two of the most influential CpG units, with ±2 SE, grouped by the treatment (CRT and TAU). Right: Box plots showing the mean methylation levels of CpG units within LC1, with ±2 SE, grouped by the treatment (CRT and TAU). These metrics are calculated separately for CpG units exhibiting decreased Δm values in the CRT compared to TAU ("negative change average") and those demonstrating the opposite trend, increased Δm values in the CRT compared to TAU ("positive change average"). Statistical significance is denoted as: $p < 0.001$ ***, $p < 0.01$ **, $p < 0.05$ *. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the LC scores, the "negative change average", and the "positive change average" to distinguish between CRT-I and the rest of the sample (CRT-NI/TAU). To control for the possibility of significant *p*-values happening by chance, as we performed 6 comparisons for each of the six domains, we only considered those significant effects that met the Bonferroni criterion ($p = 0.05 / 6 = 0.008$).

2.6.4. Interplay between NRN1 methylation profile and genetic variants on CRT effects

We analyzed the influence of LC scores, "negative change average," and "positive change average," along with NRN1 polymorphisms (11 SNPs), as combined predictors of cognitive improvement. For each cognitive domain and total cognition, three logistic regression models were developed with CRT-I versus the rest as the dependent variable, using i) cognitive scores and 11 SNPs, ii) "negative change average" and 11 SNPs, or iii) "positive change average" and 11 SNPs as independent variables. Each logistic regression was performed separately for the six cognitive domains and global cognition, resulting in a total of 21 logistic regression analyses. A stepwise approach using a conditional method was employed to include SNPs based on the likelihood-ratio statistic's probability. Due to genotype frequency, SNPs were dichotomized in all analyses (homozygous and heterozygous for the minor allele versus homozygous for the major allele).

3. Results

3.1. CRT effects on cognition

As described by the previous study by Penadés et al., 2024, from which our sample is drawn, we observed significant improvements following the intervention in five out of six cognitive domains in the CRT group compared to the TAU group: speed processing ($p = 0.002$), attention/vigilance ($p = 0.013$), working memory ($p = 0.007$), verbal learning ($p = 0.019$), and visual learning ($p = 0.031$). Additionally, the CRT group exhibited favorable differences in global cognition ($p < 0.001$) (**Supplementary Table 6**).

3.2. NRN1 methylation changes

3.2.1. NRN1 methylation changes over time between CRT and TAU

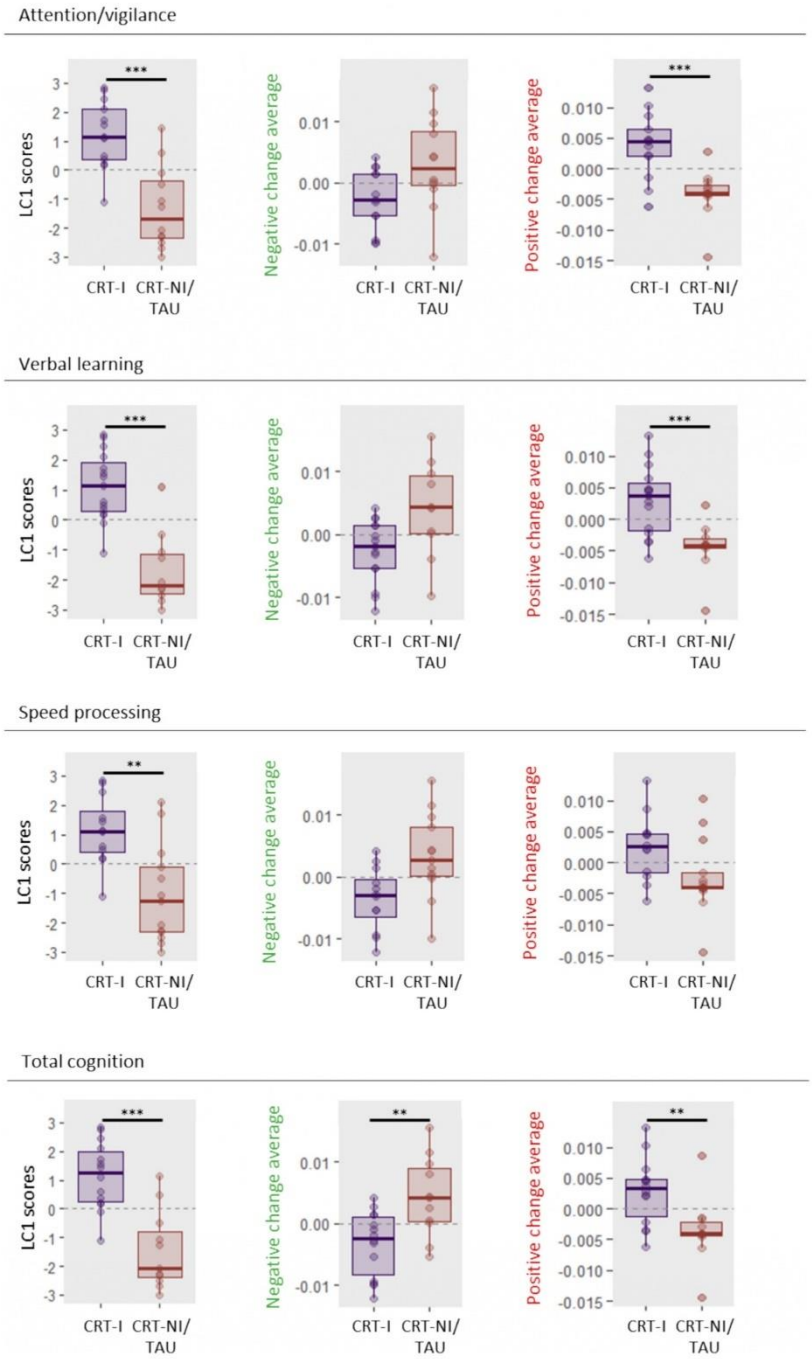
Two LCs, accounting for 6.5 % and 6.4 % of the methylation variance and containing 25 and 15 CpG units respectively, effectively differentiated between CRT and TAU. This discrimination is evidenced by the 0.28 balanced classification error rate and depicted in Fig. 2A, which illustrates the sample distribution within the two LC spaces. The performance evaluation suggests that the model including both LCs is relatively effective at identifying CRT, with an accuracy of 68.5 %, and is moderately successful at minimizing false positives and false negatives, achieving a precision rate of 68.4 % and a recall of 67.7 %, respectively. These rates result in an F1 score of 68.1 %, indicating a balanced trade-off between precision and recall. Due to the intrinsic methodology of sPLS-DA, LC1 was the most influential component in distinguishing between treatment arms. This was further confirmed by comparing the LCs scores between CRT and TAU, which showed a significant difference in LC1 (TAU = −1.97(0.83), CRT = 1.11(1.11), $U = 2$, $p < 0.001$), whereas LC2 did not reach statistical significance (TAU = 0.62(0.91), CRT = −0.35(1.50), $U = 40$, $p = 0.074$), as evidenced in Fig. 2B. Additionally, LC1 demonstrated greater stability in its features, with the 88 % of the CpG units (22 out of 25) exhibiting high stability (>85 %), in contrast to LC2, where only the 40 % of the units (6 out of 15) met this criterion (**Supplementary Table S7**). Accordingly, LC2 was considered less reliable and its features less informative compared to those in LC1. Therefore, subsequent analyses exploring methylation patterns among the CpG units were directed toward LC1. Within LC1, the included CpG units exhibited varying relevance in discriminating between treatment arms, as depicted in the loadings plot (Fig. 2C), and distinct patterns of methylation changes between the CRT and TAU groups. Specifically, a subset of CpG units exhibited lower Δm values in the CRT group compared to TAU, indicating a shift toward decreased methylation levels from baseline to post-treatment, either through a decline or a smaller increase, as depicted by green bars in Fig. 2C, and exemplified by CpG2.15 in Fig. 2D **(left)**. The Δm mean of these CpG units (listed as CpG2.15, CpG3.8, CpG1.20, CpG2.16, CpG2.30, CpG1.13, CpG2.13, CpG2.20, CpG1.19, CpG2.25, CpG2.17, CpG3.5, CpG3.11, CpG3.7, CpG2.19, CpG2.24, and CpG2.26), referred to as the "negative change average," significantly differed between groups (TAU = 0.006(0.006), CRT = -0.003(0.005), $U = 20$, $p = 0.002$, Fig. 2D **right**). Conversely, another subset of CpG units within LC1 exhibited an opposite trend, showing an increase in methylation levels from baseline to post-treatment in the CRT group compared to TAU, through an elevation or a smaller decrease, as indicated by red bars in Fig. 2C and exemplified by CpG2.22 in Fig. 2E **(left)**. The Δm mean of these CpG units (listed as CpG2.22, CpG2.21, CpG1.8, CpG1.14, CpG2.14, CpG2.8, CpG1.17, and CpG1.10), referred to as the "positive change average," also significantly differed between groups (TAU = -0.005(0.004), CRT = 0.003(0.005), $U = 12$, $p < 0.001$, Fig. 2D **right**). Some of the CpG units included in the LC1 also presented differences between CRT and TAU groups when examined individually (**Supplementary Table S8**).

3.2.2. NRN1 methylation changes over time and cognitive improvement after CRT

The scores of LC1 exhibited significant differences between patients showing a better performance after CRT (CRT-I) and the rest of the sample (encompassing those that did not improved (CRT-NI) or were in the TAU group), across domains such as attention/vigilance (CRT-NI/TAU = -1.312(1.421), CRT-I = 1.21(1.168), $U = 15.00$, $p < 0.001$), verbal learning (CRT-NI/TAU = -1.662(1.243), CRT-I = 1.108(1.144), $U = 9.00$, $p < 0.001$), and speed processing (CRT-NI/TAU = -1.05 (1.674), CRT-I = 1.13(1.167), $U = 24.00$, $p = 0.002$), as well as in overall cognitive functioning (CRT-NI/TAU = -1.46(1.36), CRT-I = 1.15 (1.17), $U = 14.00$, $p < 0.001$). Additionally, the "positive change average" also significantly differentiated CRT-I from the rest of the sample across attention/vigilance (CRT-NI/TAU = -0.004(0.004), CRT-I = 0.004(0.005), $U = 18$, $p < 0.001$), verbal learning (CRT-NI/TAU = -0.004(0.004), CRT-I = 0.003(0.006), $U = 18$, $p < 0.001$), and global cognition (CRT-NI/TAU = -0.004(0.005), CRT-I = 0.003(0.005), $U = 28$, $p = 0.006$). Conversely, the "negative change average" did not exhibit significant differences across any cognitive domains (**Supplementary Table S9**) but did so in total cognition (CRT-NI/TAU = 0.004 (0.007), CRT-I = -0.003(0.005), $U = 29$, $p = 0.008$). These findings are visually summarized in Fig. 3.

3.3. Interplay between NRN1 methylation profile and genetic variants on CRT effects

We identified significant additive effects between LC1 and rs9405890 concerning speed processing CRT improvement. Initially, the first step of the logistic regression revealed significant effects of LC1 (odds ratio OR[95% CI] = 2.570[1.211–5.465], Nagelkerke $R^2 = 0.452$, $p = 0.014$). Subsequently, in the second step, which integrated the 11 SNPs, the optimal model for discriminating between CRT-I and the rest of the sample comprised LC1 and rs9405890 (LC1 OR[95 % CI] = 4.993 [1.094–22.246], $p = 0.038$; rs9405890 OR[95 % CI] = 0.016[0–0.853], $p = 0.041$). The omnibus test yielded a significant result, indicating the overall significance of the model ($\chi^2 = 17.665$, $p < 0.001$). Notably, the inclusion of rs9405890 significantly improved the model's performance (Nagelkerke $R^2 = 0.695$, p of the change = 0.001). Upon examining the distribution of rs9405890, we observed that the 83 % of improvers were C allele carriers, compared to 38 % in the rest of the sample.



(caption on next page)

Fig. 3. Box plots illustrating various methylation metrics with ±2 standard errors (SE), categorized by cognitive improvement status: individuals undergoing cognitive remediation therapy (CRT) who showed improvement (CRT-I, dark purple) versus non-improvers (CRT-NI) and those receiving treatment as usual (TAU) (grouped as CRT-NI/TAU, dark red). The first column shows the latent component 1 (LC1) scores. The second column displays the mean methylation levels for CpG units with decreased Δm values in the CRT group compared to the TAU group, labeled "negative change average". The third column presents the mean methylation levels for CpG units with increased Δm values, labeled "positive change average". Statistical significance is indicated as follows: $p < 0.001$ ***, $p < 0.01$ **, $p < 0.05$ *. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Drawing on insights from animal and human studies emphasizing the central role of the *NRN1* gene in neuronal plasticity and cognition-related processes, we have investigated its longitudinal changes in peripheral methylation following cognitive stimulation through CRT. Our study finds that CRT is associated with *NRN1* methylation changes of specific CpG units that also correlate with improvements in cognitive performance achieved by the intervention. Additionally, we observe that incorporating data on genetic variability in *NRN1* strengthens this correlation.

Initially, we evaluated the efficacy of CRT in our sample, noting significant cognitive improvements in patients undergoing CRT compared to TAU. These results align with previous studies, including our own work (Penadés et al., 2024) and other research highlighting CRT's positive effects on cognitive performance in SZ patients (Garrido et al., 2017; Reeder et al., 2017; Vita et al., 2021).

Additionally, exploring the idea that CRT-induced cognitive recovery could be linked to changes in peripheral methylation of crucial genes, particularly those involved in synaptic plasticity, such as *RELN* and *BDNF* (Ho et al., 2020; Penadés et al., 2024), we focused on *NRN1* blood methylation shifts. Believing that both broad methylation patterns and site-specific analyses are crucial for understanding the complex etiology of SZ, we employed a multi-level strategy, starting with a global approach (derived from the PLS method) and progressing into more detailed interpretations.

First, our sPLS-DA model identified methylation changes in a set of CpG units that efficiently distinguish between CRT and TAU groups. These results suggest that *NRN1* peripheral methylation is dynamic and responsive to CRT, consistent with previous research on DNA methylation's responsiveness to behavioral stimuli (Levenson et al., 2006; Miller and Sweatt, 2007). The component that best differentiated the two treatment arms, LC1, included both CpG units with increased and decreased methylation levels post-CRT, indicating that changes in both directions are relevant to the therapy. To test the robustness of these patterns and pursuing a deeper understanding of the biological background of these differential methylation levels, we grouped CpG units based on whether they showed increased or decreased methylation and calculated the mean for each group. Both averages differed significantly between treatment arms, confirming that the observed differences were not merely data-driven but reflected a potentially meaningful biological variation.

Subsequently, we explored whether therapy-induced methylation changes correlated with therapy-derived cognitive enhancement. We found that CpG units associated with the therapy could also differentiate between CRT participants who experienced cognitive improvement from the rest of the patients. This suggests that methylation variability relates not only to treatment but also to its cognitive benefits. Specifically, LC1 scores and the "positive change average" could distinguish improvers in attention/vigilance and verbal learning, while improvements in processing speed were correlated with LC1 and not with the positive or negative averages. Despite these differences in specific domains, overall cognitive functioning was linked to all three methylation metrics.

By combining these levels of analysis, we found that while both hypermethylation and hypomethylation are associated with therapy, CpG units showing increased methylation are linked to cognitive improvement across more domains than those with decreased methylation. This seems to suggest that hypermethylation may be more strongly associated with the cognitive improvements observed after CRT. This is aligned with previous research reporting that *NRN1* methylation differences in SZ were significant only when considering the combined effect of adjacent CpGs, with hypomethylation across the locus being more relevant to the disorder than changes at individual CpG sites (Pidsley et al., 2014).

It is noteworthy that research into DNA methylation and prognosis following psychological therapy is nascent, with limited findings to date (Gooding, 2022). As mentioned earlier, previous studies have examined *RELN* and *BDNF* (Ho et al., 2020; Penadés et al., 2024), but none have explored *NRN1* methylation until now, highlighting the novelty of our focus. Nevertheless, both preceding studies are significant for *NRN1*, as animal models show a direct correlation between *BDNF* and *NRN1* expression, with *BDNF* injections in neonatal rat pups upregulating *NRN1* (Wibrand et al., 2006). Although findings have been inconsistent, Penadés et al. reported a link between *BDNF* methylation and cognitive improvement from CRT and Ho et al. observed this association with *RELN* methylation, not *BDNF*. Then, these previous results, along with our findings related to *NRN1*, suggest that changes in peripheral gene methylation involved in synaptic plasticity may be a key molecular outcome of CRT and underscore the need for further investigations to uncover potential biomarkers of CRT effectiveness.

Finally, recognizing that each CpG site could play a distinct role depending on its genomic context, we attempted to explore the functional implications of *NRN1* methylation variability. However, this interpretation is challenging due to two primary factors. First, there is a current lack of understanding regarding the effects of specific CpG units on *NRN1* gene expression. Second, divergent effects of changes in methylation levels on expression have been described, induction and repression, depending on the genomic context (Li and Zhang, 2014; Rauluseviciute et al., 2020). However, to gain insight into this issue, we employed the UCSC Genome Browser and accessed data from the Encyclopedia of DNA Elements (ENCODE) project and the Open Regulatory Annotation (ORegAnno) database. This approach facilitated the identification of regulatory elements overlapping with CpG units within LC1 (**Supplementary Table S10**). The presence of enhancer regions, DNase I hypersensitive sites, and histone marks such as H3K27me3, H3K4me1, and H3K36me3, along with target regions for various transcription factors (TF), highlight the potential role of these CpG units in gene expression control and chromatin accessibility. Focusing on the CpG units categorized under the "positive change average", which are particularly interesting for their association with cognitive improvement across multiple domains, many of them may influence the interaction of numerous TF previously implicated in SZ. CpG1.8 is a potential target for ZNF816, differentially expressed in the blood of twins discordant for depression (Zhu et al., 2019), and NFKB1, whose variants are linked to SZ risk and antipsychotic response (Long et al., 2022). CpG1.10 may affect FOXO1, sensitive to olanzapine in postmortem brain and blood (Gu et al., 2021), and ELK1, differentially expressed in blood samples from patients with SZ (Sanders et al., 2013). CpG1.17 might alter the union of ZIC2, differentially expressed in early-onset SZ cases (Xu et al., 2016) and causing SZ-like phenotypes in mutant mice (Hatayama et al., 2011). CpG2.8 might modulate the affinity of NRF1, reduced in the cortex of subjects with SZ (McMeekin et al., 2016). Finally, CpG2.14, CpG2.21 and CpG2.22 might change the effects of *EGR1*, an immediate early gene expressed in response to stress and genetically associated with SZ (Marballi and Gallitano, 2018). Therefore, we can speculate that

methylation changes in these CpG units might be directly impacting *NRN1* expression through the regulation of the affinity of different TF.

Expanding the focus on *NRN1* beyond the human-based evidence, research on cellular and animal models within the central nervous system has also shown a growing interest in *NRN1* as a potential target for cognitive therapies like CRT. Increased Nrn1 expression is linked to enhanced cognitive performance in mice, including improved spatial learning, memory recovery after ischemia-reperfusion injury, better neurological scores after traumatic brain injury, and reduced cognitive impairments in Alzheimer's models (Liu et al., 2018; Reshetnikov et al., 2020; Wan et al., 2020). Additionally, *NRN1* responds to neurotherapeutic agents such as electroconvulsive therapy, fluoxetine, and clozapine, which alter its expression through epigenetic pathways histone deacetylations (Alme et al., 2007; Park et al., 2014). The findings from these studies, along with our results, advocate for further investigation into *NRN1* as a therapeutic target in SZ. However, caution is necessary when extrapolating our findings, given that the association between *NRN1* methylation and CRT-induced cognitive improvements is based on peripheral samples. Notwithstanding, recent evidence suggests that psychotherapy can influence neuronal methylation, with these changes potentially being reflected in the blood (Quevedo et al., 2022; Turecki and Meaney, 2016).

Lastly, as literature suggests that the outcomes of psychological therapies may be influenced by both methylation and genetic variability (Bosia et al., 2014; Penadés et al., 2018; Spangaro et al., 2018), we expanded our analysis to examine genotypic variability and its interplay with methylation data. While previous studies reported the genetic-epigenetic interplay of *FKBP5* in response to cognitive behavioral therapy for anxiety (Roberts et al., 2019), our genotyping analyses suggest that *NRN1* polymorphisms additively, rather than interactively, contribute to cognitive improvement after CRT. We observed additive effects between LC1 and the rs9405890 allele on processing speed improvement following CRT. Our results further suggested that carriers of the C allele may have a higher likelihood of improving processing speed after CRT. This aligns with previous studies linking the C-A alleles of the rs9405890-rs14755157 haplotype to better cognitive function and later onset of SZ (Chandler et al., 2010; Fatjó-Vilas et al., 2016). This result suggests that, although cognitive therapies might exert their effects through plastic changes in methylation, the genetic variability of the individual also plays an important role in the response.

This study has several limitations, including a relatively small sample size, which may impact the replication of the sPLS-DA model results. However, we addressed this issue by employing a rigorous approach, incorporating leave-one-out cross-validation (LOOCV) at each stage of the modeling process, and reducing the number of features using the LASSO penalty. Despite the favorable performance metrics obtained, they may not be sufficient to accurately predict treatment responders versus non-responders, suggesting that the inclusion of additional markers or genes may be necessary to enhance the search for reliable biomarkers. Furthermore, our sample size may limit the power to detect SNP effects, their interactions with methylation, and potential sex-specific differences. Nonetheless, our sample size is comparable to other studies utilizing latent component approaches in schizophrenia research with similar biological data (Carvalho et al., 2024; Fernandez-Egea et al., 2016; Hashimoto et al., 2018). While our results are promising, they emphasize the need for larger studies to further elucidate the role of methylation changes in *NRN1* during cognitive interventions. Additionally, patients were on antipsychotic medication, which, despite adjustments, remains a potential confounding factor. Besides, other environmental factors, along with the tissue-specific and time-dependent nature of methylation processes, pose challenges. Also, while peripheral methylation levels do not directly reflect central nervous system methylation, peripheral DNA is necessary for biomarker studies in living patients. Lastly, our findings are limited to associations between SNP/methylation levels of one gene and CRT effects on cognitive improvement, but these results should be viewed in the context of the complex interactions among multiple genes and environmental factors influencing cognitive functioning.

In conclusion, we found an association between CRT and changes in *NRN1* peripheral methylation, with cognitive improvements linked to these changes. Moreover, incorporating data on *NRN1* genetic variability strengthens this correlation. Parallelly, while not our primary focus, our study adds to the widely accepted notion that cognitive remediation is an effective treatment for enhancing cognitive functioning in SZ. Thus, our integrated model, incorporating genetics, epigenetics, and cognition, advances the comprehension of CRT response variability and suggests *NRN1* as a potential peripheral biomarker for assessing CRT effectiveness.

Ethical statement

Prior to participation, all individuals provided written consent after receiving comprehensive information regarding the study procedures and potential implications. This research adhered to the guidelines set forth by the respective institutions involved and obtained approval from the local ethics committee of the participating centers, in strict accordance with the principles outlined in the Declaration of Helsinki. All ethical regulations pertinent to research involving human participants were strictly followed throughout the entirety of this study.

CRediT authorship contribution statement

Carmen Almodóvar-Payá: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Irene París-Gómez:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Mariona Latorre-Guardia:** Writing – review & editing, Methodology, Formal analysis. **Maria Guardiola-Ripoll:** Writing – review & editing, Investigation. **Rosa Catalán:** Writing – review & editing, Investigation, Data curation. **Bárbara Arias:** Writing – review & editing, Funding acquisition, Conceptualization. **Rafael Penadés:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Mar Fatjó-Vilas:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

Declaration of competing interest Rafael Penadés has received honoraria/travel support (unrelated to the present work) from Angelini. The rest of the authors reported no biomedical financial interests or potential conflicts of interest.

Acknowledgements

This work was supported by: i) Fundación Alicia Koplowitz, ii) Acadèmia de les Ciències Mèdiques i de la Salut de Catalunya i de Balears, iii) the NARSAD Independent investigator grant (Project 24618), and Fondo de Investigaciones Sanitarias (FIS), Ministerio de Economía y Competitividad, Instituto de Salud Carlos III (PI17/00872; Fondo Europeo de Desarrollo Regional, Unión Europea, "Una manera de hacer Europa"). This study also received funding provided by: i) the Instituto de Salud Carlos III through a Miguel Servet contract to MF–V (CP20/00072), co-funded by European Regional Development Fund (ERDF)/European Social Fund "Investing in your future"; (ii) the Acadèmia de les Ciències Mèdiques i de la Salut de Catalunya i de Balears through a contract to CA-P; iii) the Comissionat per a Universitats i Recerca del DIUE of the Generalitat de Catalunya, Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR: 2021SGR1475, 2021SGR00706).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2024.111175.

References

Aberg, K.A., McClay, J.L., Nerella, S., Clark, S., Kumar, G., Chen, W., Khachane, A.N., Xie, L., Hudson, A., Gao, G., Harada, A., Hultman, C.M., Sullivan, P.F., Magnusson, P. K.E., van den Oord, E.J.C.G., 2014. Methylome-wide association study of schizophrenia: identifying blood biomarker signatures of environmental insults. JAMA Psychiatry 71 (3), 255–264. https://doi.org/10.1001/jamapsychiatry.2013.3730.

Alme, M.N., Wibrand, K., Dagestad, G., Bramham, C.R., 2007. Chronic fluoxetine treatment induces brain region-specific upregulation of genes associated with BDNF-induced long-term potentiation. Neural Plast. 2007, 26496. https://doi.org/10.1155/2007/26496.

Almodóvar-Payá, C., Guardiola-Ripoll, M., Giralt-López, M., Gallego, C., Salgado-Pineda, P., Miret, S., Salvador, R., Muñoz, M.J., Lázaro, L., Guerrero-Pedraza, A., Parellada, M., Carrión, M.I., Cuesta, M.J., Maristany, T., Sarró, S., Fañanás, L., Callado, L.F., Arias, B., Pomarol-Clotet, E., Fatjó-Vilas, M., 2022. NRN1 gene as a potential marker of early-onset schizophrenia: evidence from genetic and neuroimaging approaches. Int. J. Mol. Sci. 23 (13), 13. https://doi.org/10.3390/ijms23137456.

Almodóvar-Payá, C., Guardiola-Ripoll, M., Giralt-López, M., Oscoz-Irurozqui, M., Canales-Rodríguez, E.J., Madre, M., Soler-Vidal, J., Ramiro, N., Callado, L.F., Arias, B., Gallego, C., Pomarol-Clotet, E., Fatjó-Vilas, M., 2024 Jun. NRN1 epistasis with BDNF and CACNA1C: mediation effects on symptom severity through neuroanatomical changes in schizophrenia. Brain Struct Funct 229 (5), 1299–1315. https://doi.org/10.1007/s00429-024-02793-5.

Bora, E., 2017. A comparative meta-analysis of neurocognition in first-degree relatives of patients with schizophrenia and bipolar disorder. Eur. Psychiatry 45, 121–128. https://doi.org/10.1016/j.eurpsy.2017.06.003.

Bora, E., Lin, A., Wood, S.J., Yung, A.R., McGorry, P.D., Pantelis, C., 2014. Cognitive deficits in youth with familial and clinical high risk to psychosis: a systematic review and meta-analysis. Acta Psychiatr. Scand. 130 (1), 1–15. https://doi.org/10.1111/acps.12261.

Bosia, M., Bechi, M., Pirovano, A., Buonocore, M., Lorenzi, C., Cocchi, F., Bramanti, P., Smeraldi, E., Cavallaro, R., 2014. COMT and 5-HT1A-receptor genotypes potentially affect executive functions improvement after cognitive remediation in schizophrenia. Health Psychol. Behav. Med. 2 (1), 509–516. https://doi.org/10.1080/21642850.2014.905206.

Carvalho, M.S., Sforca, M.L., Rocco, S.A., Furtado, D.Z.S., da Silverio, C.S., Nunes, M.F. Q., Vella Nunes, M., Adelino, M.P.M., Afonso dos Santos, L., Jackowski, A., Affonseca Bressan, R., Lacerda, A.L.T., Assunção, N.A., 2024. Integration of proteinogenic amino acids in the pathogenesis of schizophrenia. Med. Adv. 2 (2), 123–135. https://doi.org/10.1002/med4.65.

Chan, R.F., Shabalin, A.A., Montano, C., Hannon, E., Hultman, C.M., Fallin, M.D., Feinberg, A.P., Mill, J., van den Oord, E.J.C.G., Aberg, K.A., 2020. Independent methylome-wide association studies of schizophrenia detect consistent case–control differences. Schizophr. Bull. 46 (2), 319–327. https://doi.org/10.1093/schbul/sbz056.

Chandler, D., Dragović, M., Cooper, M., Badcock, J.C., Mullin, B.H., Faulkner, D., Wilson, S.G., Hallmayer, J., Howell, S., Rock, D., Palmer, L.J., Kalaydjieva, L., Jablensky, A., 2010. Impact of Neuritin 1 (NRN1) polymorphisms on fluid intelligence in schizophrenia. Am. J. Med. Genet. B Neuropsychiatr. Genet. 153B (2), 428–437. https://doi.org/10.1002/ajmg.b.30996.

Charlson, F.J., Ferrari, A.J., Santomauro, D.F., Diminic, S., Stockings, E., Scott, J.G., McGrath, J.J., Whiteford, H.A., 2018. Global epidemiology and burden of schizophrenia: findings from the global burden of disease study 2016. Schizophr. Bull. 44 (6), 1195–1203. https://doi.org/10.1093/schbul/sby058.

Ciuculete, D.M., Boström, A.E., Voisin, S., Philipps, H., Titova, O.E., Bandstein, M., Nikontovic, L., Williams, M.J., Mwinyi, J., Schiöth, H.B., 2017. A methylome-wide mQTL analysis reveals associations of methylation sites with GAD1 and HDAC3 SNPs and a general psychiatric risk score. Transl. Psychiatry 7 (1), e1002. https://doi.org/10.1038/tp.2016.275.

Davies, M.N., Volta, M., Pidsley, R., Lunnon, K., Dixit, A., Lovestone, S., Coarfa, C., Harris, R.A., Milosavljevic, A., Troakes, C., Al-Sarraj, S., Dobson, R., Schalkwyk, L.C., Mill, J., 2012. Functional annotation of the human brain methylome identifies tissue-specific epigenetic variation across brain and blood. Genome Biol. 13 (6), R43. https://doi.org/10.1186/gb-2012-13-6-r43.

Dempster, E.L., Pidsley, R., Schalkwyk, L.C., Owens, S., Georgiades, A., Kane, F., Kalidindi, S., Picchioni, M., Kravariti, E., Toulopoulou, T., Murray, R.M., Mill, J., 2011. Disease-associated epigenetic changes in monozygotic twins discordant for schizophrenia and bipolar disorder. Hum. Mol. Genet. 20 (24), 4786–4796. https://doi.org/10.1093/hmg/ddr416.

Doig, G.S., Simpson, F., 2005. Randomization and allocation concealment: a practical guide for researchers. J. Crit. Care 20 (2), 187–191 discussion 191-193. https://doi.org/10.1016/j.jcrc.2005.04.005.

Eack, S.M., Hogarty, G.E., Cho, R.Y., Prasad, K.M.R., Greenwald, D.P., Hogarty, S.S., Keshavan, M.S., 2010. Neuroprotective effects of cognitive enhancement therapy against gray matter loss in early schizophrenia: results from a 2-year randomized controlled trial. Arch. Gen. Psychiatry 67 (7), 674–682. https://doi.org/10.1001/archgenpsychiatry.2010.63.

Fatjó-Vilas, M., Prats, C., Pomarol-Clotet, E., Lázaro, L., Moreno, C., González-Ortega, I., Lera-Miguel, S., Miret, S., Muñoz, M.J., Ibáñez, I., Campanera, S., Giralt-López, M., Cuesta, M.J., Peralta, V., Ortet, G., Parellada, M., González-Pinto, A., McKenna, P.J., Fañanás, L., 2016. Involvement of NRN1 gene in schizophrenia-spectrum and bipolar disorders and its impact on age at onset and cognitive functioning. World J. Biol. Psychiatry 17 (2), 129–139. https://doi.org/10.3109/15622975.2015.1093658.

Fatouros-Bergman, H., Cervenka, S., Flyckt, L., Edman, G., Farde, L., 2014. Meta-analysis of cognitive performance in drug-naïve patients with schizophrenia. Schizophr. Res. 158 (1), 156–162. https://doi.org/10.1016/j.schres.2014.06.034.

Fernandez-Egea, E., Vértes, P.E., Flint, S.M., Turner, L., Mustafa, S., Hatton, A., Smith, K. G.C., Lyons, P.A., Bullmore, E.T., 2016. Peripheral immune cell populations associated with cognitive deficits and negative symptoms of treatment-resistant schizophrenia. PLoS ONE 11 (5), e0155631. https://doi.org/10.1371/journal.pone.0155631.

First, M., Spitzer, R., Gibbon, M., Williams, J., 2002. Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition. En (SCID-I/P).

Fisher, H.L., Murphy, T.M., Arseneault, L., Caspi, A., Moffitt, T.E., Viana, J., Hannon, E., Pidsley, R., Burrage, J., Dempster, E.L., Wong, C.C.Y., Pariante, C.M., Mill, J., 2015. Methylomic analysis of monozygotic twins discordant for childhood psychotic symptoms. Epigenetics 10 (11), 1014–1023. https://doi.org/10.1080/15592294.2015.1099797.

Flavell, S.W., Greenberg, M.E., 2008. Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. Annu. Rev. Neurosci. 31, 563–590. https://doi.org/10.1146/annurev.neuro.31.060407.125631.

Garrido, G., Penadés, R., Barrios, M., Aragay, N., Ramos, I., Vallès, V., Faixa, C., Vendrell, J.M., 2017. Computer-assisted cognitive remediation therapy in schizophrenia: durability of the effects and cost-utility analysis. Psychiatry Res. 254, 198–204. https://doi.org/10.1016/j.psychres.2017.04.065.

Gooding, D.C., 2022. Brave New World: harnessing the promise of biomarkers to help solve the epigenetic puzzle. Schizophr. Res. 242, 35–41. https://doi.org/10.1016/j.schres.2022.01.020.

Goud Alladi, C., Etain, B., Bellivier, F., Marie-Claire, C., 2018. DNA methylation as a biomarker of treatment response variability in serious mental illnesses: A systematic review focused on bipolar disorder, schizophrenia, and major depressive disorder. Int. J. Mol. Sci. 19, 10. https://doi.org/10.3390/ijms19103026.

Gu, S., Cui, F., Yin, J., Fang, C., Liu, L., 2021. Altered mRNA expression levels of autophagy- and apoptosis-related genes in the FOXO pathway in schizophrenia patients treated with olanzapine. Neurosci. Lett. 746, 135669. https://doi.org/10.1016/j.neulet.2021.135669.

Hallmayer, J.F., Kalaydjieva, L., Badcock, J., Dragović, M., Howell, S., Michie, P.T., Rock, D., Vile, D., Williams, R., Corder, E.H., Hollingsworth, K., Jablensky, A., 2005. Genetic evidence for a distinct subtype of schizophrenia characterized by pervasive cognitive deficit. Am. J. Hum. Genet. 77 (3), 468–476. https://doi.org/10.1086/432816.

Hannon, E., Dempster, E., Viana, J., Burrage, J., Smith, A.R., Macdonald, R., St Clair, D., Mustard, C., Breen, G., Therman, S., Kaprio, J., Toulopoulou, T., Pol, H.E.H., Bohlken, M.M., Kahn, R.S., Nenadic, I., Hultman, C.M., Murray, R.M., Collier, D.A., Mill, J., 2016. An integrated genetic-epigenetic analysis of schizophrenia: evidence for co-localization of genetic associations and differential DNA methylation. Genome Biol. 17 (1), 176. https://doi.org/10.1186/s13059-016-1041-x.

Hashimoto, R., Itahashi, T., Okada, R., Hasegawa, S., Tani, M., Kato, N., Mimura, M., 2018. Linked functional network abnormalities during intrinsic and extrinsic activity in schizophrenia as revealed by a data-fusion approach. NeuroImage 17, 69–79. https://doi.org/10.1016/j.nicl.2017.09.025.

Hatayama, M., Ishiguro, A., Iwayama, Y., Takashima, N., Sakoori, K., Toyota, T., Nozaki, Y., Odaka, Y.S., Yamada, K., Yoshikawa, T., Aruga, J., 2011. Zic2 hypomorphic mutant mice as a schizophrenia model and ZIC2 mutations identified in schizophrenia patients. Sci. Rep. 1, 16. https://doi.org/10.1038/srep00016.

Hilker, R., Helenius, D., Fagerlund, B., Skytthe, A., Christensen, K., Werge, T.M., Nordentoft, M., Glenthøj, B., 2018. Heritability of schizophrenia and schizophrenia spectrum based on the Nationwide Danish twin register. Biol. Psychiatry 83 (6), 492–498. https://doi.org/10.1016/j.biopsych.2017.08.017.

Ho, N.F., Tng, J.X.J., Wang, M., Chen, G., Subbaraju, V., Shukor, S., Ng, D.S.X., Tan, B.-L., Puang, S.J., Kho, S.-H., Siew, R.W.E., Sin, G.L., Eu, P.W., Zhou, J., Sng, J.C.G., Sim, K., Medalia, A., 2020. Plasticity of DNA methylation, functional brain connectivity and efficiency in cognitive remediation for schizophrenia. J. Psychiatr. Res. 126, 122–133. https://doi.org/10.1016/j.jpsychires.2020.03.013.

Hurst, C., Pugh, D.A., Abreha, M.H., Duong, D.M., Danmer, E.B., Bennett, D.A., Herskowitz, J.H., Seyfried, N.T., 2023. Integrated proteomics to understand the role of Neuritin (NRN1) as a mediator of cognitive resilience to Alzheimer's disease. Mol. Cell. Proteomics 22 (5). https://doi.org/10.1016/j.mcpro.2023.100542.

Iasevoli, F., Giordano, S., Balletta, R., Latte, G., Formato, M.V., Prinzivalli, E., De Berardis, D., Tomasetti, C., de Bartolomeis, A., 2016. Treatment resistant schizophrenia is associated with the worst community functioning among severely-ill highly-disabling psychiatric conditions and is the most relevant predictor of poorer achievements in functional milestones. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 65, 34–48. https://doi.org/10.1016/j.pnpbp.2015.08.010.

Jaenisch, R., Bird, A., 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat. Genet. 33 (3), 245–254. https://doi.org/10.1038/ng1089.

Javaherian, A., Cline, H.T., 2005. Coordinated motor neuron axon growth and neuromuscular synaptogenesis are promoted by CPG15 in vivo. Neuron 45 (4), 505–512. https://doi.org/10.1016/j.neuron.2004.12.051.

Jirtle, R.L., Skinner, M.K., 2007. Environmental epigenomics and disease susceptibility. Nat. Rev. Genet. 8 (4), 253–262. https://doi.org/10.1038/nrg2045.

Johnson, E.C.B., Carter, E.K., Dammer, E.B., Duong, D.M., Gerasimov, E.S., Liu, Y., Liu, J., Betarbet, R., Ping, L., Yin, L., Serrano, G.E., Beach, T.G., Peng, J., De Jager, P. L., Haroutunian, V., Zhang, B., Gaiteri, C., Bennett, D.A., Gearing, M., Seyfried, N.T., 2022. Large-scale deep multi-layer analysis of Alzheimer's disease brain reveals strong proteomic disease-related changes not observed at the RNA level. Nat. Neurosci. 25 (2), 213–225. https://doi.org/10.1038/s41593-021-00999-y.

Jonas, K., Lian, W., Callahan, J., Ruggero, C.J., Clouston, S., Reichenberg, A., Carlson, G. A., Bromet, E.J., Kotov, R., 2022. The course of general cognitive ability in individuals with psychotic disorders. JAMA Psychiatry 79 (7), 659–666. https://doi.org/10.1001/jamapsychiatry.2022.1142.

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 13 (2), 261–276. https://doi.org/10.1093/schbul/13.2.261.

Kebir, O., Chaumette, B., Rivollier, F., Miozzo, F., Lemieux Perreault, L.P., Barhdadi, A., Provost, S., Plaze, M., Bourgin, J., Gaillard, R., Mezger, V., Dubé, M.-P., Krebs, M.-O., 2017. Methylomic changes during conversion to psychosis. Mol. Psychiatry 22 (4), 512–518. https://doi.org/10.1038/mp.2016.53.

Kern, R.S., Nuechterlein, K.H., Green, M.F., Baade, L.E., Fenton, W.S., Gold, J.M., Keefe, R.S.E., Mesholam-Gately, R., Mintz, J., Seidman, L.J., Stover, E., Marder, S.R., 2008. The MATRICS consensus cognitive battery, part 2: co-norming and standardization. Am. J. Psychiatry 165 (2), 214–220. https://doi.org/10.1176/appi.ajp.2007.07010043.

Khavari, B., Cairns, M.J., 2020. Epigenomic dysregulation in schizophrenia: in search of disease etiology and biomarkers. Cells 9, 8. https://doi.org/10.3390/cells9081837.

Levenson, J.M., Roth, T.L., Lubin, F.D., Miller, C.A., Huang, I.-C., Desai, P., Malone, L.M., Sweatt, J.D., 2006. Evidence that DNA (Cytosine-5) methyltransferase regulates synaptic plasticity in the Hippocampus *. J. Biol. Chem. 281 (23), 15763–15773. https://doi.org/10.1074/jbc.M511767200.

Li, E., Zhang, Y., 2014. DNA methylation in mammals. Cold Spring Harb. Perspect. Biol. 6 (5), a019133. https://doi.org/10.1101/cshperspect.a019133.

Liu, Q., Zhang, H., Xu, J., Zhao, D., 2018. Neuritin provides neuroprotection against experimental traumatic brain injury in rats. Int. J. Neurosci. 128 (9), 811–820. https://doi.org/10.1080/00207454.2018.1424155.

Loebrich, S., Nedivi, E., 2009. The function of activity-regulated genes in the nervous system. Physiol. Rev. 89 (4), 1079–1103. https://doi.org/10.1152/physrev.00013.2009.

Long, J., Tian, L., Baranova, A., Cao, H., Yao, Y., Rao, S., Zhang, F., 2022. Convergent lines of evidence supporting involvement of *NFKB1* in schizophrenia. Psychiatry Res. 312, 114588. https://doi.org/10.1016/j.psychres.2022.114588.

Marballi, K.K., Gallitano, A.L., 2018. Immediate early genes anchor a biological pathway of proteins required for memory formation, long-term depression and risk for schizophrenia. Front. Behav. Neurosci. 12. https://doi.org/10.3389/fnbeh.2018.00023.

Matsuoka, K., Morimoto, T., Matsuda, Y., Yasuno, F., Taoka, T., Miyasaka, T., Yoshikawa, H., Takahashi, M., Kitamura, S., Kichikawa, K., Kishimoto, T., 2019. Computer-assisted cognitive remediation therapy for patients with schizophrenia induces microstructural changes in cerebellar regions involved in cognitive functions. Psychiatry Res. Neuroimaging 292, 41–46. https://doi.org/10.1016/j.pscychresns.2019.09.001.

McMeekin, L.J., Lucas, E.K., Meador-Woodruff, J.H., McCullumsmith, R.E., Hendrickson, R.C., Gamble, K.L., Cowell, R.M., 2016. Cortical PGC-1α-dependent transcripts are reduced in postmortem tissue from patients with schizophrenia. Schizophr. Bull. 42 (4), 1009–1017. https://doi.org/10.1093/schbul/sbv184.

Michalopoulou, P.G., Lewis, S.W., Wykes, T., Jaeger, J., Kapur, S., 2013. Treating inpaired cognition in schizophrenia: the case for combining cognitive-enhancing drugs with cognitive remediation. Eur. Neuropsychopharmacol. 23 (8), 790–798. https://doi.org/10.1016/j.euroneuro.2013.03.012.

Miller, C.A., Sweatt, J.D., 2007. Covalent modification of DNA regulates memory formation. Neuron 53 (6), 857–869. https://doi.org/10.1016/j.neuron.2007.02.022.

Montano, C., Taub, M.A., Jaffe, A., Briem, E., Feinberg, J.I., Trygvadottir, R., Idrizi, A., Runarsson, A., Berndsen, B., Gur, R.C., Moore, T.M., Perry, R.T., Fugman, D., Sabunciyan, S., Yolken, R.H., Hyde, T.M., Kleinman, J.E., Sobell, J.L., Pato, C.N., Feinberg, A.P., 2016. Association of DNA methylation differences with schizophrenia in an epigenome-wide association study. JAMA Psychiatry 73 (5), 506–514. https://doi.org/10.1001/jamapsychiatry.2016.0144.

Moore, L.D., Le, T., Fan, G., 2013. DNA methylation and its basic function. Neuropsychopharmacology 38 (1), 23–38. https://doi.org/10.1038/npp.2012.112.

Morgan, S.E., Seidlitz, J., Whitaker, K.J., Romero-Garcia, R., Clifton, N.E., Scarpazza, C., van Amelsvoort, T., Marcelis, M., van Os, J., Donohoe, G., Mothersill, D., Corvin, A., Pocklington, A., Raznahan, A., McGuire, P., Vértes, P.E., Bullmore, E.T., 2019. Cortical patterning of abnormal morphometric similarity in psychosis is associated with brain expression of schizophrenia-related genes. Proc. Natl. Acad. Sci. 116 (19), 9604–9609. https://doi.org/10.1073/pnas.1820754116.

Morimoto, T., Matsuda, Y., Matsuoka, K., Yasuno, F., Ikebuchi, E., Kameda, H., Taoka, T., Miyasaka, T., Kichikawa, K., Kishimoto, T., 2018. Computer-assisted cognitive remediation therapy increases hippocampal volume in patients with schizophrenia: a randomized controlled trial. BMC Psychiatry 18 (1), 83. https://doi.org/10.1186/s12888-018-1667-1.

Mothersill, D., Donohoe, G., 2019. Neural effects of cognitive training in schizophrenia: a systematic review and activation likelihood estimation meta-analysis. Biol. Psychiatry Cogn. Neurosci. Neuroimag. 4 (8), 688–696. https://doi.org/10.1016/j.bpsc.2019.03.005.

Nedivi, E., Fieldust, S., Theill, L.E., Hevron, D., 1996. A set of genes expressed in response to light in the adult cerebral cortex and regulated during development. Proc. Natl. Acad. Sci. USA 93 (5), 2048–2053.

Nielsen, R.E., Levander, S., Kjaersdam Telléus, G., Jensen, S.O.W., Østergaard Christensen, T., Leucht, S., 2015. Second generation antipsychotic effect on cognition in patients with schizophrenia—A meta-analysis of randomized clinical trials. Acta Psychiatr. Scand. 131 (3), 185–196. https://doi.org/10.1111/acps.12374.

Nuechterlein, K.H., Green, M.F., Kern, R.S., Baade, L.E., Barch, D.M., Cohen, J.D., Essock, S., Fenton, W.S., Frese, F.J., Gold, J.M., Goldberg, T., Heaton, R.K., Keefe, R. S.E., Kraemer, H., Mesholam-Gately, R., Seidman, L.J., Stover, E., Weinberger, D.R., Young, A.S., Marder, S.R., 2008. The MATRICS consensus cognitive battery, part 1: test selection, reliability, and validity. Am. J. Psychiatry 165 (2), 203–213. https://doi.org/10.1176/appi.ajp.2007.07010042.

Park, H.G., Yu, H.S., Park, S., Ahn, Y.M., Kim, Y.S., Kim, S.H., 2014. Repeated treatment with electroconvulsive seizures induces HDAC2 expression and down-regulation of NMDA receptor-related genes through histone deacetylation in the rat frontal cortex. Int. J. Neuropsychopharmacol. 17 (9), 1487–1500. https://doi.org/10.1017/S1461145714000248.

Penadés, R., Pujol, N., Catalán, R., Massana, G., Rametti, G., García-Rizo, C., Bargalló, N., Gastó, C., Bernardo, M., Junqué, C., 2013. Brain effects of cognitive remediation therapy in schizophrenia: a structural and functional neuroimaging study. Biol. Psychiatry 73 (10), 1015–1023. https://doi.org/10.1016/j.biopsych.2013.01.017.

Penadés, R., González-Rodríguez, A., Catalán, R., Segura, B., Bernardo, M., Junqué, C., 2017. Neuroimaging studies of cognitive remediation in schizophrenia: a systematic and critical review. World J. Psychiatry 7 (1), 34–43. https://doi.org/10.5498/wjp.v7.i1.34.

Penadés, R., López-Vílchez, I., Catalán, R., Arias, B., González-Rodríguez, A., García-Rizo, C., Masana, G., Ruíz, V., Mezquida, G., Bernardo, M., 2018. BDNF as a marker of response to cognitive remediation in patients with schizophrenia: a randomized and controlled trial. Schizophr. Res. 197, 458–464. https://doi.org/10.1016/j.schres.2017.12.002.

Penadés, R., Arias, B., Fatjó-Vilas, M., González-Vallespí, L., García-Rizo, C., Catalán, R., Bernardo, M., 2020a. Epigenetic studies in psychotherapy: a systematic review. Curr. Psychiatry Res. Rev. 16 (2), 86–92. https://doi.org/10.2174/2666082216999200622140922.

Penadés, R., Segura, B., Inguanzo, A., García-Rizo, C., Catalán, R., Masana, G., Bernardo, M., Junqué, C., 2020b. Cognitive remediation and brain connectivity: a resting-state fMRI study in patients with schizophrenia. Psychiatry Res. Neuroimaging 303, 111140. https://doi.org/10.1016/j.pscychresns.2020.111140.

Penadés, R., Almodóvar-Payá, C., García-Rizo, C., Ruíz, V., Catalán, R., Valero, S., Wykes, T., Fatjó-Vilas, M., Arias, B., 2024. Changes in *BDNF* methylation patterns after cognitive remediation therapy in schizophrenia: a randomized and controlled trial. J. Psychiatr. Res. 173, 166–174. https://doi.org/10.1016/j.jpsychires.2024.03.014.

Perera, B.P.U., Faulk, C., Svoboda, L.K., Goodrich, J.M., Dolinoy, D.C., 2020. The role of environmental exposures and the epigenome in health and disease. Environ. Mol. Mutagen. 61 (1), 176–192. https://doi.org/10.1002/em.22311.

Petronis, A., Gottesman, I.I., Kan, P., Kennedy, J.L., Basile, V.S., Paterson, A.D., Popendikyte, V., 2003. Monozygotic twins exhibit numerous epigenetic differences: clues to twin discordance? Schizophr. Bull. 29 (1), 169–178. https://doi.org/10.1093/oxfordjournals.schbul.a006988.

Pidsley, R., Viana, J., Hannon, E., Spiers, H., Troakes, C., Al-Saraj, S., Mechawar, N., Turecki, G., Schalkwyk, L.C., Bray, N.J., Mill, J., 2014. Methylomic profiling of human brain tissue supports a neurodevelopmental origin for schizophrenia. Genome Biol. 15 (10), 483. https://doi.org/10.1186/s13059-014-0483-2.

Posthuma, D., Luciano, M., de Geus, E.J.C., Wright, M.J., Slagboom, P.E., Montgomery, G.W., Boomsma, D.I., Martin, N.G., 2005. A Genomewide scan for intelligence identifies quantitative trait loci on 2q and 6p. Am. J. Hum. Genet. 77 (2), 318–326.

Prats, C., Arias, B., Ortet, G., Ibáñez, M.I., Moya, J., Ponarol-Clotet, E., Fañanás, L., Fatjó-Vilas, M., 2017. Role of neurotrophins in depressive symptoms and executive function: association analysis of NRN1 gene and its interaction with BDNF gene in a non-clinical sample. J. Affect. Disord. 211, 92–98. https://doi.org/10.1016/j.jad.2016.11.017.

Putz, U., Harwell, C., Nedivi, E., 2005. Soluble CPG15 expressed during early development rescues cortical progenitors from apoptosis. Nat. Neurosci. 8 (3), 322–331. https://doi.org/10.1038/nn1407.

Quevedo, Y., Booij, L., Herrera, L., Hernández, C., Jiménez, J.P., 2022. Potential epigenetic mechanisms in psychotherapy: a pilot study on DNA methylation and mentalization change in borderline personality disorder. Front. Hum. Neurosci. 16, 955005. https://doi.org/10.3389/fnhum.2022.955005.

Ramsay, I.S., Nienow, T.M., Marggraf, M.P., MacDonald, A.W., 2017. Neuroplastic changes in patients with schizophrenia undergoing cognitive remediation: triple-blind trial. Br. J. Psychiatry 210 (3), 216–222. https://doi.org/10.1192/bjp.bp.115.171496.

Rauluseviciute, I., Drabløs, F., Rye, M.B., 2020. DNA hypermethylation associated with upregulated gene expression in prostate cancer demonstrates the diversity of epigenetic regulation. BMC Med. Genet. 13 (1), 6. https://doi.org/10.1186/s12920-020-0657-6.

Reeder, C., Wykes, T., 2010. Computerised Interactive Remediation of Cognition–Interactive Training for Schizophrenia (CIRCUITS), London. Kings College. https://scholar.google.com/scholar_lookup?hl=en&publication_year=2010&author=C.+Reeder&author=T.+Wykes&title=Computerised+interactive+remediation+of+cognition%E2%80%94Interactive+training+for+schizophrenia+%28CIRCUITS%29.

Reeder, C., Huddy, V., Cella, M., Taylor, R., Greenwood, K., Landau, S., Wykes, T., 2017. A new generation computerised metacognitive cognitive remediation programme for

schizophrenia (CIRCuiTS): a randomised controlled trial. Psychol. Med. 47 (15), 2720–2730. https://doi.org/10.1017/S0033291717001234.
Reshetnikov, V.V., Kisaretova, P.E., Ershov, N.I., Shulyupova, A.S., Oshchepkov, D.Y., Klimova, N.V., Ivanchihina, A.V., Merkulova, T.I., Bondar, N.P., 2020. Genes associated with cognitive performance in the Morris water maze: an RNA-seq study. Sci. Rep. 10 (1), 22078. https://doi.org/10.1038/s41598-020-78997-6.
Roberts, S, Keers, R, Breen, G, Coleman, JRI, Jöhren, P, Kepa, A, Lester, KJ, Margraf, J, Scheider, S, Teismann, T, Wannemüller, A, Eley, TC, Wong, CCY, 2019 Mar. DNA methylation of FKBP5 and response to exposure-based psychological therapy. Am J Med Genet B Neuropsychiatr Genet 180 (2), 150–158. https://doi.org/10.1002/ajmg.b.32650.
Rohart, F., Gautier, B., Singh, A., Cao, K.-A.L., 2017. mixOmics: an R package for 'omics feature selection and multiple data integration. PLoS Comput. Biol. 13 (11), e1005752. https://doi.org/10.1371/journal.pcbi.1005752.
Sanders, A.R., Göring, H.H.H., Duan, J., Drigalenko, E.I., Moy, W., Freda, J., He, D., Shi, J., Gejman, P.V., 2013. Transcriptome study of differential expression in schizophrenia. Hum. Mol. Genet. 22 (24), 5001–5014. https://doi.org/10.1093/hmg/ddt350.
Schmitt, A., Falkai, P., Papiol, S., 2023. Neurodevelopmental disturbances in schizophrenia: evidence from genetic and environmental factors. J. Neural Transm. 130 (3), 195–205. https://doi.org/10.1007/s00702-022-02567-5.
Schübeler, D., 2015. Function and information content of DNA methylation. Nature 517 (7534), 321–326. https://doi.org/10.1038/nature14192.
Shah, S., McRae, A.F., Marioni, R.E., Harris, S.E., Gibson, J., Henders, A.K., Redmond, P., Cox, S.R., Pattie, A., Corley, J., Murphy, L., Martin, N.G., Montgomery, G.W., Starr, J.M., Wray, N.R., Deary, I.J., Visscher, P.M., 2014. Genetic and environmental exposures constrain epigenetic drift over the human life course. Genome Res. 24 (11), 1725–1733. https://doi.org/10.1101/gr.176933.114.
Spangaro, M., Bosia, M., Bechi, M., Buonocore, M., Cocchi, F., Guglielmino, C., Bianchi, L., Mastromatteo, A., Lorenzi, C., Cavallaro, R., 2018. Neurobiology of cognitive remediation in schizophrenia: effects of EAAT2 polymorphism. Schizophr. Res. 202, 106–110. https://doi.org/10.1016/j.schres.2018.06.059.
Sullivan, P.F., Kendler, K.S., Neale, M.C., 2003. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch. Gen. Psychiatry 60 (12), 1187. https://doi.org/10.1001/archpsyc.60.12.1187.
Trubetskoy, V., Pardiñas, A.F., Qi, T., Panagiotaropoulou, G., Awasthi, S., Bigdeli, T.B., Bryois, J., Chen, C.-Y., Dennison, C.A., Hall, L.S., Lam, M., Watanabe, K., Frei, O., Ge, T., Harwood, J.C., Koopmans, F., Magnusson, S., Richards, A.L., Sidorenko, J., O'Donovan, M.C., 2022. Mapping genomic loci implicates genes and synaptic biology in schizophrenia. Nature 604, 7906. https://doi.org/10.1038/s41586-022-04434-5.
Turecki, G., Meaney, M., 2016. Effects of the social environment and stress on glucocorticoid receptor gene methylation: a systematic review. Biol. Psychiatry 79 (2), 87–96. https://doi.org/10.1016/j.biopsych.2014.11.022.
Vita, A., Barlati, S., Ceraso, A., Nibbio, G., Ariu, C., Deste, G., Wykes, T., 2021. Effectiveness, core elements, and moderators of response of cognitive remediation for schizophrenia. JAMA Psychiatry 78 (8), 1–12. https://doi.org/10.1001/jamapsychiatry.2021.0620.
Wagner, J.R., Busche, S., Ge, B., Kwan, T., Pastinen, T., Blanchette, M., 2014. The relationship between DNA methylation, genetic and expression inter-individual variation in untransformed human fibroblasts. Genome Biol. 15 (2), R37. https://doi.org/10.1186/gb-2014-15-2-r37.
Walton, E., Hass, J., Liu, J., Roffman, J.L., Bernardoni, F., Roessner, V., Kirsch, M., Schackert, G., Calhoun, V., Ehrlich, S., 2016. Correspondence of DNA methylation between blood and brain tissue and its application to schizophrenia research. Schizophr. Bull. 42 (2), 406–414. https://doi.org/10.1093/schbul/sbv074.
Wan, K., Mao, F., Li, Q., Wang, L., Wei, Z., Wang, P., Liao, X., Xu, M., Huang, J., Pan, Z., Wang, C., Luo, J., Gao, R., Gan, S., 2020. Neuritin-overexpressing transgenic mice demonstrate enhanced neuroregeneration capacity and improved spatial learning and memory recovery after ischemia-reperfusion injury. Aging (Albany NY) 13 (2), 2681–2699. https://doi.org/10.18632/aging.202318.
Wibrand, K., Messaoudi, E., Håvik, B., Steenslid, V., Løvlie, R., Steen, V.M., Bramham, C. R., 2006. Identification of genes co-upregulated with arc during BDNF-induced long-term potentiation in adult rat dentate gyrus in vivo. Eur. J. Neurosci. 23 (6), 1501–1511. https://doi.org/10.1111/j.1460-9568.2006.04687.x.
Wykes, T., Brammer, M., Mellers, J., Bray, P., Reeder, C., Williams, C., Corner, J., 2002. Effects on the brain of a psychological treatment: cognitive remediation therapy: functional magnetic resonance imaging in schizophrenia. Br. J. Psychiatry 181 (2), 144–152. https://doi.org/10.1192/bjp.181.2.144.
Xu, Y., Yao Shugart, Y., Wang, G., Cheng, Z., Jin, C., Zhang, K., Wang, J., Yu, H., Yue, W., Zhang, F., Zhang, D., 2016. Altered expression of mRNA profiles in blood of early-onset schizophrenia. Sci. Rep. 6 (1), 16767. https://doi.org/10.1038/srep16767.
Yu, L., Tasaki, S., Schneider, J.A., Arfanakis, K., Duong, D.M., Wingo, A.P., Wingo, T.S., Kearns, N., Thatcher, G.R.J., Seyfried, N.T., Levey, A.I., De Jager, P.L., Bennett, D.A., 2020. Cortical proteins associated with cognitive resilience in community-dwelling older persons. JAMA Psychiatry 77 (11), 1172–1180. https://doi.org/10.1001/jamapsychiatry.2020.1807.
Zhu, Y., Strachan, E., Fowler, E., Bacus, T., Roy-Byrne, P., Zhao, J., 2019. Genome-wide profiling of DNA methylome and transcriptome in peripheral blood monocytes for major depression: a monozygotic discordant twin study. Transl. Psychiatry 9, 215. https://doi.org/10.1038/s41398-019-0550-2.

5. Summary of the Results

The three proposed hypotheses were explored through aligned objectives, organized into sections, which resulted in the four articles presented, with their results summarized below.

In relation to **HYPOTHESIS 1** "*NRN1 genetic variability will be associated with an increased risk of schizophrenia, particularly in early-onset forms of the disorder. The neurobiological basis of this association will be evidenced by the role of NRN1 gene in the functional and structural brain differences observed in patients compared to healthy subjects and also by its modulation of clinical manifestations. In addition, these effects will be further shaped by epistatic effects between NRN1 and other molecularly related genes, such as BDNF and CACNA1C",* the objectives and results were organized into section I.

Section 1: The role of NRN1 genetic variability in schizophrenia risk, intermediate phenotypes, clinical expression, and its interactions with related genes

The two specific objectives delineated resulted in two scientific articles:

I.I. Almodóvar-Payá C, et al. *NRN1* gene as a potential marker of early-onset schizophrenia: evidence from genetic and neuroimaging approaches. *Int J Mol Sci*. 2022 Jul 5;23(13):7456. doi: 10.3390/ijms23137456.

I.II. Almodóvar-Payá C, et al. *NRN1* epistasis with *BDNF* and *CACNA1C*: mediation effects on symptom severity through neuroanatomical changes in schizophrenia. *Brain Struct Funct*. 2024 Jun;229(5):1299-1315. doi: 10.1007/s00429-024-02793-5.

In these two scientific articles, the following **results** have been obtained:

I.I. We identified a *NRN1* three-SNP haplotype, including rs3763180, rs10484320, and rs4960155, that was associated with the risk for early-onset schizophrenia spectrum disorders (EO SSD) in two independent samples (case-control and family-based). In EO families, the GCT haplotype was significantly under-transmitted from parents to affected offspring. In the case-control sample, the G allele of rs3763180, the T allele of rs10484320, and the T allele of rs4960155 were significantly associated with EO SSD under an additive model. Similarly, the GTT haplotype was more frequent in individuals with EO SSD, while the TCC haplotype was more prevalent in controls, supporting the SNP-based findings. No associations were found between *NRN1* variability and adult-onset SSD risk in any allelic, genotypic, or haplotypic analyses. Additionally, in the neuroimaging genetics approach, a significant interaction between diagnosis and the GTT haplotype was observed in the 2-back vs. 1-back contrast in a cluster located in the superior and middle frontal gyrus, regions of the dorsolateral prefrontal cortex (DLPFC). EO patients without the risk haplotype showed increased activity in this cluster from 1-back to 2-back contrasts, whereas those carrying the risk haplotype exhibited a decrease in cluster activity.

I.II. We did not find any epistatic effects between *NRN1* and *BDNF*-rs6265 or *NRN1* and *CACNA1C*-rs1006737 on schizophrenia risk. However, we identified a significant gene-gene combined effect of *NRN1*-rs10484320 and *BDNF*-rs6265 on PANSS general psychopathology scores, and a notable *NRN1*-rs4960155 x *CACNA1C*-rs1006737 interaction on GAF scores. Additionally, we observed several significant *NRN1* x *BDNF*-rs6265 interactions affecting the cortical surface area in frontal, parietal, and temporal regions, as well as cortical volume in frontal regions. Similarly, *NRN1* x *CACNA1C*-rs1006737 interactions significantly influenced the cortical surface area of temporal regions and cortical volume in frontal regions. Finally, we observed that the *NRN1*-rs10484320 x *BDNF*-rs6265 epistasis in the left lateral orbitofrontal cortex fully mediated the effect on PANSS general psychopathology.

Regarding the **HYPOTHESIS II** "*NRN1 methylation and expression levels in the brain will be correlated and will differ between individuals with schizophrenia and control subjects. These differences will be modulated by antipsychotic treatment and influenced by genetic variability within the locus*", the objectives and results were organized into section II.

Section II: Brain NRN1 genetic, epigenetic, and expression correlates and the modulatory effect of antipsychotic treatment

The specific objective delineated resulted in one scientific article:

II. Almodóvar-Payá et al., 2024. Clozapine-related brain *NRN1* expression patterns are associated with methylation and genetic variants in schizophrenia. *Submitted for peer review in an indexed journal.*

In this scientific article, the following **results** have been obtained:

II. We found that clozapine-treated schizophrenia patients had lower *NRN1* mRNA levels in the prefrontal cortex compared to both anti-psychotic free schizophrenia patients and controls. Additionally, clozapine-treated schizophrenia patients presented distinct methylation patterns across multiple CpG units in both regions compared to controls, while untreated patients exhibited a pattern closer to controls. Using sparse Partial Least Squares-Discriminant Analysis (sPLS-DA), which combines dimensionality reduction, feature selection, and classification, we identified three latent components in the prefrontal cortex and two in the hippocampus differentiating between clozapine-treated patients and controls, with both regions achieving a classification error rate of 0.28, validated through Leave-One-Out Cross-Validation (LOOCV). The most discriminative component in both models, LC1, included CpG units with both increased and decreased methylation levels, suggesting that changes in both directions contributed to group differences. However, the CpG units differentiating the groups varied between the two brain regions, indicating distinct *NRN1* epigenetic patterns. In the prefrontal cortex, the CpG units differentiating clozapine-treated

schizophrenia patients from controls, particularly those in LC1 and the subset showing decreased methylation, were correlated to *NRN1* expression in that region. Lastly, we found that genetic variability within the *NRN1* locus influenced these effects, as the impact of LC1 and the subset showing decreased methylation in clozapine-treated patients on expression was enhanced by considering the *NRN1*-rs12333117 and *NRN1*-rs2208870 polymorphisms.

Concerning the **HYPOTHESIS III** "*NRN1 peripheral epigenetic changes following Cognitive Remediation Therapy (CRT) will be associated with cognitive improvements, with this effect further influenced by genetic variability within the locus*", the objectives and results were organized into section III.

Section III: The impact of NRN1 genetic variability and peripheral epigenetic changes on cognitive improvements following Cognitive Remediation Therapy (CRT)

The specific objective resulted in one scientific article:

III. Almodóvar-Payá et al. *NRN1* genetic variability and methylation changes as biomarkers for cognitive remediation therapy response in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*. 2024 Oct 17:111175. doi: 10.1016/j.pnpbp.2024.111175. Epub ahead of print.

In this scientific article, the following **results** have been obtained:

III. We initially evaluated the efficacy of CRT in our sample and found significant cognitive improvements in patients undergoing CRT compared to those receiving TAU. Furthermore, we identified significant differences in *NRN1* methylation across multiple CpG units between the two treatment arms, suggesting that *NRN1* peripheral methylation is dynamic and responsive to CRT. Using sparse Partial Least Squares-Discriminant Analysis (sPLS-DA), which combines dimensionality reduction, feature selection, and classification, we identified two latent components that differentiated CRT from TAU, achieving a classification error rate of 0.28, validated through Leave-One-Out Cross-Validation (LOOCV). Within the most discriminative component, LC1, we identified CpG units with both increased and decreased methylation levels post-CRT, highlighting that methylation changes in both directions play a role in the therapy's effects. Additionally, CpG units associated with therapy were linked to cognitive improvements, with LC1 and the subset of CpG units showing increased post-therapy methylation distinguishing CRT responders from the rest of the patients across multiple cognitive domains, suggesting that hypermethylation may play a more prominent role in the therapy's effects. Lastly, we demonstrated that genetic variability within the *NRN1* locus influenced these effects, as the impact of LC1 on speed processing improvement after CRT was enhanced when accounting for the *NRN1*-rs9405890 polymorphism.

6. Discussion

Framework of the present thesis

This thesis is grounded in the field of psychiatric genetics and aims to advance our understanding of the complex etiological factors contributing to schizophrenia. As a multifaceted disorder, schizophrenia imposes substantial personal, economic, and social burdens. Despite significant advancements in research, the precise causes and pathophysiological mechanisms remain largely elusive, and, importantly, no reliable biological markers have been identified to guide diagnosis or treatment. Consequently, current therapeutic approaches are often accompanied by considerable side effects and demonstrate variable efficacy across patients.

While it is true that the past decade has seen considerable progress in unraveling the genetics of schizophrenia, primarily due to large-scale consortia and whole-genome studies, these efforts face a significant challenge in addressing the clinical heterogeneity of the disorder. Additionally, while these studies are not hypothesis-driven and allow for the identification of genes and molecular pathways with high statistical power, they are not directed to explain how these genes modulate the phenotype. To address this, it is essential to work with more homogeneous patient samples and to explore how these genetic variants contribute to the disorder beyond their statistical association with disease risk.

We have approached this challenge by using intermediate phenotypes in the study of the *NRN1* gene, which serves as a key example of genes implicated in schizophrenia due to its dual role in brain development and synaptic plasticity in the mature brain. Our approach involved an integrative study combining molecular and neuroimaging techniques, offering a comprehensive perspective on how *NRN1* may contribute to the disorder.

The state of the art before the thesis

Prior to this thesis, substantial knowledge about the role of *NRN1* in the brain was established, primarily through animal and cell-based studies, as detailed in the introduction. In summary, *NRN1* is known to regulate apoptosis in proliferative neurons, promote neuronal migration and synaptic maturation, modulate neurite outgrowth during differentiation, stimulate dendritic arbor growth, stabilize active synapses, and regulate synaptic plasticity (Zhou & Zhou, 2014). Furthermore, its neuroprotective properties were demonstrated in several studies. For example, overexpression of *NRN1* rescues muscular atrophy in zebrafish by protecting neuromuscular axons, promotes axonal regeneration after facial nerve injury in hamsters, and enhances axonal regeneration and restores hindlimb function following spinal cord injury in rats through exogenous application (Shimada et al., 2013).

More importantly, there was also compelling evidence supporting the role of *NRN1* in psychiatric disorders. In animal models, increased *Nrn1* expression has been linked to reduced cognitive impairments in Alzheimer's models, while decreased expression has

been associated with depression (An et al., 2014; Son et al., 2012). Additionally, *Nrn1* responds to neurotherapeutic interventions, such as electroconvulsive therapy and fluoxetine, which modulate its expression through epigenetic mechanisms, including histone deacetylation (Alme et al., 2007; Dyrvig et al., 2014; Newton et al., 2003; Park et al., 2014). Even more significantly, human studies have implicated the *NRN1* gene in mental disorder risk, early onset of symptoms, and cognitive deficits in patients (Chandler et al., 2010; Fatjó-Vilas et al., 2016). Additionally, it emerged as one of the most differentially methylated genes in the prefrontal cortex of schizophrenia patients compared to controls (Pidsley et al., 2014).

Moreover, during the course of the present thesis, further studies appeared emphasizing the role of *NRN1* in cognition. For example, increased *Nrn1* expression has been linked to improved cognitive performance in mice, including enhanced spatial learning, memory recovery after ischemia-reperfusion injury, and better neurological outcomes following traumatic brain injury (Liu et al., 2018; Reshetnikov et al., 2020; Wan et al., 2020). Simultaneously, evidence from human post-mortem prefrontal cortex samples supports these findings, showing that NRN1 is among the most highly expressed proteins in elderly individuals with cognitive resilience, further suggesting a neuroprotective role for *NRN1* (Hurst et al., 2023; Yu et al., 2020).

Despite progress, significant gaps remained in our understanding of the role of *NRN1* in schizophrenia, and the precise mechanisms through which it contributed to the onset and progression of the disorder had yet to be fully clarified. Among other questions, we lacked detailed information on whether *NRN1* exhibited altered expression in schizophrenia, which regulatory mechanisms might be involved, and whether *NRN1* or its pathways were associated with alterations in brain structure or function, or in clinical presentation. Additionally, it was still unclear whether *NRN1* could influence the response to specific treatments. Our work has contributed to addressing these gaps, which is crucial for improving our understanding of the role of *NRN1* in the disorder and for translating genetic findings into effective diagnostic and therapeutic strategies.

Contributions of the thesis to the field of psychiatric genetics

With a focus on NRN1

To investigate the role of *NRN1* in schizophrenia, in this thesis we adopted a multilevel approach, which resulted in four articles reflecting the progression of our efforts clarifying this complex issue. First, we assessed whether genetic variants of *NRN1* were associated with schizophrenia risk and related phenotypes, including neuroimaging and clinical traits. We then explored whether these effects were modulated by other genes potentially involved in *NRN1* molecular networks. Next, building on these promising findings, we expanded our analysis to post-mortem brain samples to determine whether the observed effects on neuroimaging and symptoms profile were driven by differences in brain

expression, methylation, and genetic variability. Finally, we explored whether changes in peripheral *NRN1* methylation, along with individual genetic variability, could account for differences in response to cognitive remediation therapy.

Then, our first objective was to investigate the extent to which common DNA sequence variants in the *NRN1* gene influence the modulation of schizophrenia phenotypes. To achieve this, we utilized genetic association approaches, including both family-based and case-control designs. In addition, we examined genotypic effects using multiple strategies, ranging from single-SNP analyses to more complex models, such as additive genetic effects through haplotype structures involving multiple SNPs within *NRN1*, as well as gene-gene interactions by assessing epistasis between *NRN1* variants and those of its putative molecular interactors. Additionally, we explore their impact, not only in relation to clinical outcomes, but also to neuroimaging phenotypes.

Thus, our initial two publications show that the *NRN1* polymorphic variability, independently and in combination with *BDNF*-rs6265 or *CACN1C*-rs1006737, is closely linked to the clinical heterogeneity observed in schizophrenia-spectrum disorder patients.

On the one hand, by integrating family-based and case-control analyses, we identified that three SNPs, rs3763180, rs10484320, and rs4960155, either individually or as part of distinct haplotypes, that were associated with early-onset schizophrenia, displaying both protective and risk-enhancing effects. The GCT and TCC haplotypes appeared protective, being under-transmitted to early-onset cases and more common in controls, while the G, T, and T alleles, as well as the GTT haplotype, were associated with higher risk, being more frequent in early-onset cases. Our results added to a preceding work exploring the association of *NRN1* with schizophrenia-spectrum disorders that also detected different haplotypes involving these SNPs with age at onset (Fatjó-Vilas et al., 2016). Although the specific alleles involved differed between this study and ours, this discrepancy likely stems from variations in sample populations and methodologies. Our research compared early-onset cases to controls, whereas the previous study used an intra-patient approach, treating age at onset as a quantitative trait. Despite this, both studies importantly highlight the significance of this genomic region in influencing the age of onset in schizophrenia, underscoring it as a key area for understanding the more severe forms of the disorder with poorer prognosis. Notably, different studies have linked another gene in this region, Dysbindin-1, either independently or in interaction with *NRN1*, to both schizophrenia risk and early-onset forms of the disorder (Fatjó-Vilas et al., 2011; Prats et al., 2017).

On the other hand, following an intra-patient analysis, we identified a significant interaction between *NRN1*-rs10484320 and *BDNF*-rs6265 that influenced PANSS general psychopathology, which includes symptoms like anxiety, guilt, tension, depression, and disorientation (Kay et al., 1987). Additionally, we found a significant interaction between *NRN1*-rs4960155 and *CACNA1C*-rs1006737 that affected GAF scores, which assess personal, social, and psychological functioning (Endicott et al., 1976). These findings

extend prior research on the interaction between *NRN1* and *BDNF* in schizophrenia-spectrum disorders risk and age at onset, while also offering new insights by examining epistasis in symptomatology, making this the first study to explore the interaction with *CACNA1C* (Fatjó-Vilas et al., 2016). Although our data does not align with previous findings of an epistatic association between *NRN1* and *BDNF* with increased risk for schizophrenia-spectrum disorders, this discrepancy may stem from differences in diagnostic criteria, as the earlier study used a broader diagnostic framework. Nonetheless, our results still emphasize the clinical relevance of the well-established molecular interaction between *NRN1* and *BDNF*. As regards to such interaction, BDNF binds to the TrkB receptor in the synaptic cleft, activating the transcription factor CREB, which then attaches to the *NRN1* promoter in vivo (Fujino et al., 2003). This process drives functional and structural neuronal changes, ultimately consolidating long-term synaptic plasticity (Kaldun & Sprecher, 2019). Moreover, animal studies further support this connection, showing that injecting Bdnf into neonatal rat pups increases *Nrn1* expression in vivo (Wibrand et al., 2006). In addition, our study supports the molecular link between *NRN1* and *CACNA1C* and provides new evidence suggesting its potential significance to functional impairments in schizophrenia. In this case, the expression of the *NRN1* in the prefrontal cortex through its signaling pathway mediated through the insulin receptor and ERK signaling pathways enhances synaptic transmission by increasing the surface expression of CaV1.2, CaV1.3, and CaV3.3 channels, which are essential for long-term potentiation in the processes of learning and memory (Lu et al., 2017; Yao et al., 2012; Zhao et al., 2018).

Nevertheless, given the complex etiology, pathophysiology and clinical heterogeneity of schizophrenia, it is unlikely that the genetic background directly influences the clinical presentation of the disorder. Instead, genetic networks may modulate fundamental traits that subsequently drive the manifestation of symptoms (Glahn et al., 2010; Meijer et al., 2021). Therefore, building on previous research, we suggest that breaking down schizophrenia into biologically validated and stable trait markers, such as brain structural measures, and investigating their role in mediating symptoms could lead to a clearer understanding of the disorder (Kirschner et al., 2020; Miranda et al., 2019; Sudre et al., 2020). This approach may help connect genetic variability related to synaptic plasticity with the diverse and intricate clinical manifestations of the disorder.

From one perspective, to investigate the neurobiological implications of genetic variants linked to increased risk for early-onset schizophrenia, given the clinical characteristics of these patients, we used functional MRI with the n-back task in a matched case-control subset to assess the role of *NRN1* variability on a cognitive dimension particularly affected in schizophrenia, the working memory (Wu & Jiang, 2020). Our findings showed that the GTT risk haplotype (*NRN1* rs3763180-rs10484320-rs4960155), associated with earlier onset, is linked to altered performance and brain activity during the N-back task. On the one side, early-onset patients exhibited different dorsolateral prefrontal cortex activity compared to controls, particularly when

transitioning from low to high memory-load conditions. Concretely, while controls showed stable activity in this region regardless of task difficulty and haplotype, early-onset patients with the risk haplotype exhibited reduced activity under increased load. On the other side, performance data also revealed that controls performed consistently well, while early-onset patients with the risk haplotype performed worse than those without it at low memory loads. Interpreting these results requires understanding that sustained frontoparietal activation supports high memory loads, while dorsolateral prefrontal activation is linked to the cognitive effort needed to execute the task correctly (Riley & Constantinidis, 2016; Thomas et al., 2021). Therefore, the combination of reduced activation under rising task demands and lower performance at low memory loads in early-onset patients with the risk haplotype suggests that their peak activation and performance may be reached at a lower cognitive load compared to early-onset patients without the haplotype, who appear able to recruit additional prefrontal resources as task demands increase. These findings are consistent with two previous studies demonstrating the modulatory effect of *NRN1* polymorphisms, both within our identified haplotype and others, on premorbid and current intelligence quotient in schizophrenia (Chandler et al., 2010; Fatjó-Vilas et al., 2016). Consequently, our results contribute new evidence by indicating that this cognitive impact of *NRN1* polymorphisms may be driven by their influence on brain activity.

From the other part, due to the observed epistatic interactions between *NRN1* and *BDNF* or *CACNA1C* on symptomatology, we decided to investigate the impact of these gene combinations on brain structure within patients, with the goal of demonstrating the mediation effect of the brain on these symptoms. Our findings suggest that interactions between *NRN1* SNPs and *BDNF*-rs6265 affect cortical volume and surface area in multiple brain regions, including the bilateral orbitofrontal cortex, left caudal middle frontal cortex, right lateral occipital cortex, left pars opercularis, and right postcentral gyrus. Our findings showed that the interaction between *NRN1*-rs10484320 and *BDNF*-rs6265 modulated the volume of the left lateral orbitofrontal cortex, while the rs4960155 and *BDNF*-rs6265 interaction influenced the cortical surface area and volume of the left caudal middle frontal cortex. Additionally, other *NRN1* SNPs, such as rs2208870, in combination with *BDNF*-rs6265, modulated the surface area of the right lateral occipital cortex, and rs9379002 interacted with *BDNF*-rs6265 to affect both the cortical surface area of the left pars opercularis and right postcentral gyrus, as well as the surface area and volume of the right lateral orbitofrontal cortex. However, from all those epistatic interactions affecting the brain, the one involving *NRN1*-rs10484320 and *BDNF*-rs6265 emerged as the most interesting, as it fully mediates the effect observed in PANSS general psychopathology. Specifically, patients with the lowest PANSS general psychopathology scores, specifically those with *NRN1*-rs10484320 CC and *BDNF*-rs6265 ValVal genotypes, also had the smallest left lateral orbitofrontal volume. We chose to explore the epistatic effects on brain structure due to its influence on symptomatology, rather than cognition, and the limitations of functional MRI, which primarily captures brain

activity in response to specific tasks (Riley & Constantinidis, 2016; Thomas et al., 2021). Understanding structural findings, however, requires recognizing the close relationship between structure and function. While structural organization governs neurotransmitter release and functional networks, brain activity, in turn, reshapes structure over time through neuroplasticity. For instance, research consistently shows that the resting-state network, a dynamic system, largely reflects underlying structural connectivity, indicating that structural changes can profoundly impact brain function, influencing mood, cognition, and symptom expression (Wang et al., 2020). Therefore, given the roles of the studied genes in neurodevelopment, our findings suggest that specific epistatic interactions may influence early brain formation, resulting in structural changes that subsequently modulate symptom manifestation. Interestingly, proteomic studies of the prefrontal cortex have linked NMDA receptor hypofunction and disruptions in Ca2+ homeostasis to orbitofrontal volume changes in schizophrenia (Nascimento & Martins-de-Souza, 2015; Velásquez et al., 2019). This is particularly relevant to *NRN1*, as NMDA receptor-mediated neurotransmission and plasticity are influenced by the *BDNF*-rs6265 genotype, especially in the hippocampus and infralimbic medial prefrontal cortex, and simultaneously regulate *NRN1* expression in the cortex through Ca2+ signaling. At the clinical level, reducing glutamatergic neurotransmission with NMDA receptor antagonists induces or exacerbates psychotic symptoms, highlighting the critical role of this pathway in the development of schizophrenia symptoms (Krystal et al., 1994). As a result, both neurotrophic factors, *NRN1* and *BDNF*, could influence the molecular pathways responsible for the volumetric changes in the left lateral orbitofrontal cortex, with these changes being behaviorally manifested in general psychopathology.

At this stage, we questioned whether our findings might be driven by differences in *NRN1* brain expression and methylation influenced by individual genetic variability. To explore this, we extended our analysis to post-mortem brain samples. Given the known impact of antipsychotics on the brain and previous animal studies suggesting that various drugs can modulate *NRN1* expression, we conducted the analysis by dividing patients based on the presence of clozapine in their bloodstream at the time of death, as well as those without any medications.

Hence, our third article showed unique *NRN1* epigenetic-expression correlations in the prefrontal cortex of clozapine-treated patients compared to controls, further influenced by *NRN1* polymorphisms, highlighting brain region-specific *NRN1* alterations in schizophrenia.

Clozapine-treated patients exhibited reduced expression of *NRN1* in the prefrontal cortex compared to both controls and antipsychotic-free schizophrenia patients. This finding is particularly noteworthy, as *NRN1* plays a direct role in regulating synaptic plasticity in the prefrontal cortex (Lu et al., 2017; Yao et al., 2012; Zhao et al., 2018). Interpreting these results in the context of prior studies is challenging, as ours is the first to investigate the *NRN1* expression specifically in post-mortem brain samples from

schizophrenia patients. Nonetheless, a previous study on transcriptomic profiles of the prefrontal cortex in schizophrenia patients may offer some context, as it identified two distinct molecular subgroups: one resembling the controls and another markedly different, with *NRN1* downregulated in the latter group. Yet, comparing our findings to that study is complicated because the authors did not account for the medication status of the patients (Bowen et al., 2019). Notwithstanding, in our study we also examined the expression levels of *UMHK1*, another synapse-related gene sensitive to clozapine, that similarly to *NRN1* showed reduced expression in the prefrontal cortex of clozapine-treated patients compared to controls, with no such reduction observed in the hippocampus (Rizig et al., 2012). Thus, our results provide new evidence suggesting that the reduced *NRN1* expression in the prefrontal cortex of schizophrenia patients may be specifically associated with, or at least more pronounced under, clozapine treatment.

Next, building on previous evidence identifying *NRN1* as one of the top differentially methylated genes in the prefrontal cortex of patients with schizophrenia, we explored *NRN1* methylation using a strategy that examined both global patterns and specific CpG sites. To address the intercorrelations and multicollinearity among CpG units, we applied a sparse Partial Least Squares method, allowing for dimensionality reduction, feature selection, and classification to identify methylation differences between groups, thereby enhancing the clinical relevance of the results compared to other dimensionality reduction techniques that do not account for sample labels. We found that patients with schizophrenia not treated with antipsychotics showed a methylation pattern similar to controls in both brain regions, while clozapine-treated patients exhibited a distinctly different profile. In the clozapine group, the most discriminant component included both hyper- and hypomethylated CpG units compared to controls. However, when grouped by the direction of methylation changes, only the hypomethylated CpG units showed a significant difference between patients and controls in both brain regions, suggesting that lower methylation may play a more prominent role in clozapine-treated patients in these brain areas. Interestingly, our findings from the prefrontal cortex globally align with the previously identified hypomethylated region (chr6:59992669-6006917) in the *NRN1* gene body, as reported in the mentioned whole-methylome study and further suggest that this hypomethylation may be specific to clozapine-treated patients (Pidsley et al., 2014).

But more importantly, our study provides novel insights by identifying the correlation between *NRN1* expression and methylation, encompassing both the component and average of the hypomethylated CpG units, in the prefrontal cortex but not in the hippocampus. These findings converge with previous research in animal models, which used a hypothesis-driven approach focused on early-immediate genes or genes related to plasticity, suggesting that *NRN1* is responsive to neurotherapeutic agents. For instance, both acute and chronic electroconvulsive therapy have been shown to enhance Nrn1 expression in the hippocampus, while chronic fluoxetine treatment similarly increases its levels, following a brain region-specific pattern, particularly in the prefrontal cortex and hippocampus (Alme et al., 2007; Dyrvig et al., 2014; Newton et al., 2003).

However, contrasting evidence from another study suggests that repeated electroconvulsive seizures can upregulate histone deacetylases in the rat frontal cortex, which target the downregulation of genes related to NMDA receptor signaling, including *Nrn1* (Park et al., 2014). Thus, our findings add new evidence from human post-mortem brain samples, showing that patients with schizophrenia treated with clozapine, likely reflecting chronic treatment, exhibit changes in methylation and a reduction in *NRN1* expression.

However, clozapine treatment is generally reserved for patients with persistent positive symptoms despite two adequate trials of other antipsychotics, categorizing them as treatment-resistant and possibly representing an etiological subgroup with unique biological characteristics (Howes et al., 2016). This perspective is supported by theories suggesting dopamine regulation abnormalities, such as hypersensitivity, baseline dopamine differences, and increased striatal dopamine synthesis and release probably due to disrupted GABA and glutamate balance, which overstimulates dopamine activity resulting in the positive symptoms of schizophrenia (Potkin et al., 2020). In addition, genome-wide studies associate NMDA receptor-related genes with this subgroup (Ruderfer et al., 2016). In parallel, animal studies have shown that clozapine treatment enhances NMDA receptor activation and, through its antagonist effect at dopamine D4 receptors, leads to upregulation of AMPA receptors (Tanahashi et al., 2012; Veerman et al., 2014). These genetic and molecular links are especially relevant to *NRN1*, as its expression is influenced by NMDA pathways, and it has been shown to facilitate the recruitment of AMPA receptors to synapses (Cantallops et al., 2000; Fujino et al., 2003). Consequently, our research may also contribute to understanding the etiological basis of this subgroup, providing insights that could refine treatment approaches and guide the development of novel therapeutic options.

Having come this far and based on promising evidence linking *NRN1* to cognitive resilience, we then decided to explore the potential clinical applicability of our findings by investigating whether peripheral *NRN1* methylation could be modulated by cognitive therapy, potentially serving as a biomarker for response to cognitive remediation therapy.

As a result, our fourth article highlights *NRN1* methylation changes in patients undergoing cognitive remediation therapy compared to those receiving standard treatment. More importantly, our findings showed that these methylation changes were also linked to therapy response, with the association strengthened when incorporating the rs9405890 polymorphism into the model.

In this case, to explore methylation changes, calculated by subtracting pre-treatment values from post-treatment values, we followed the same statistical approach used in the third article. Our results, derived from sparse partial least square discriminant analysis, identified methylation changes in a set of CpG units that effectively distinguished between patients undergoing cognitive remediation and those receiving standard treatment. These findings suggest that *NRN1* peripheral methylation is dynamic and

responsive to cognitive remediation therapy. Additionally, we found that CpG units associated with the therapy could also differentiate between CRT participants who experienced cognitive improvement from the rest of the patients, specifically global methylation changes and the average of CpG units experiencing an increase methylation level after the therapy, suggesting that methylation variability relates not only to treatment but also to its cognitive benefit. These results are difficult to interpret in the light of previous literature, as research into DNA methylation and prognosis following psychological therapy is nascent, with limited findings to date, and but none have explored *NRN1* methylation until now (Gooding, 2022). However, previous studies, including one from our group, have identified peripheral methylation changes linked to cognitive improvement in synaptic plasticity genes, such as *BDNF* and *RELN* (Ho et al., 2020; Penadés et al., 2024).

The inclusion of only treatment-resistant patients in this study, all of whom were treated with clozapine, further supports the potential of *NRN1* as a biomarker for this distinct etiological subgroup with specific biological characteristics.

Although our results are challenging to interpret due to being derived from peripheral blood samples, expanding our findings to data from the central nervous system reveals increasing interest in *NRN1* as a candidate for cognitive therapies. In animal models, Nrn1 has shown a protective role in various neurological conditions. In traumatic brain injury, Nrn1 injections into the injured rat cortex improved neurological scores, repaired neurons, and protected cortical cells from apoptosis (Liu et al., 2018). Similarly, in mice model of Alzheimer's disorder, brain infusion of recombinant Nrn1 rescued deficits in hippocampal long-term potentiation (An et al., 2014). Furthermore, transgenic mice overexpressing Nrn1 exhibited reduced hippocampal apoptosis, increased neuron survival, and enhanced recovery of spatial learning and memory after transient global ischemia (Wan et al., 2020). Supporting its role in cognitive function, RNA-seq analysis of high-performing mice in the Morris water maze revealed elevated expression of genes involved in glutamatergic transmission and long-term potentiation, including Nrn1 and its associated AMPA receptor signaling, which is crucial for memory (Reshetnikov et al., 2020). In neurons cultured with Aβ42, NRN1 blocks Aβ42-induced dendritic spine degeneration and protects against neuronal hyperexcitability. Furthermore, *NRN1* treatment induces changes in the neuronal proteome linked to a broad range of synaptic functions and interacts with proteins associated with cognitive resilience in the human brain (Hurst et al., 2023). On the other hand, proteomic studies conducted on human post-mortem brain samples from elderly individuals with cognitive resilience or those asymptomatic for Alzheimer's disease have identified *NRN1* as one of the top proteins associated with resilience, using hypothesis-free correlation networks (Hurst et al., 2023; Yu et al., 2020).

Overall, these results contribute significantly to the understanding of schizophrenia by providing a deeper insight into the molecular mechanisms underlying the disorder's

clinical heterogeneity. The identification of *NRN1* variability, both independently and in combination with key polymorphisms such as *BDNF*-rs6265 and *CACNA1C*-rs1006737, highlights the genetic complexity that influences schizophrenia-spectrum disorders. Furthermore, the discovery of unique *NRN1* epigenetic-expression correlations in the prefrontal cortex of clozapine-treated patients underscores the importance of region-specific molecular changes in the brain. This research also shows how *NRN1* methylation changes, particularly in response to cognitive remediation therapy, could serve as biomarkers for therapy response, offering a potential pathway for personalized treatment strategies.

With a general focus on schizophrenia specifically centered on synaptic plasticity

In line with existing literature about schizophrenia, both genomic and non-genomic findings strongly indicate that alterations in synaptic function and plasticity play a crucial role in schizophrenia. These findings highlight synaptic plasticity as a promising therapeutic target, especially considering that some of these mechanisms may be modifiable in the adult brain.

The current promise of psychiatric genomics is to offer an unbiased view into the biological basis of mental illness. However, the precise molecular mechanisms by which disorder-associated genes increase risk remain unclear, as translating genomic findings into actionable insights is inherently a complex and labor-intensive process. Therefore, the challenge now is to leverage genomic data to shed light on these critical processes and identify viable therapeutic targets. For that, we believe that a multidisciplinary and integrative approach, which assesses candidate genes across all levels of heterogeneity, is essential (**Figure 7**).

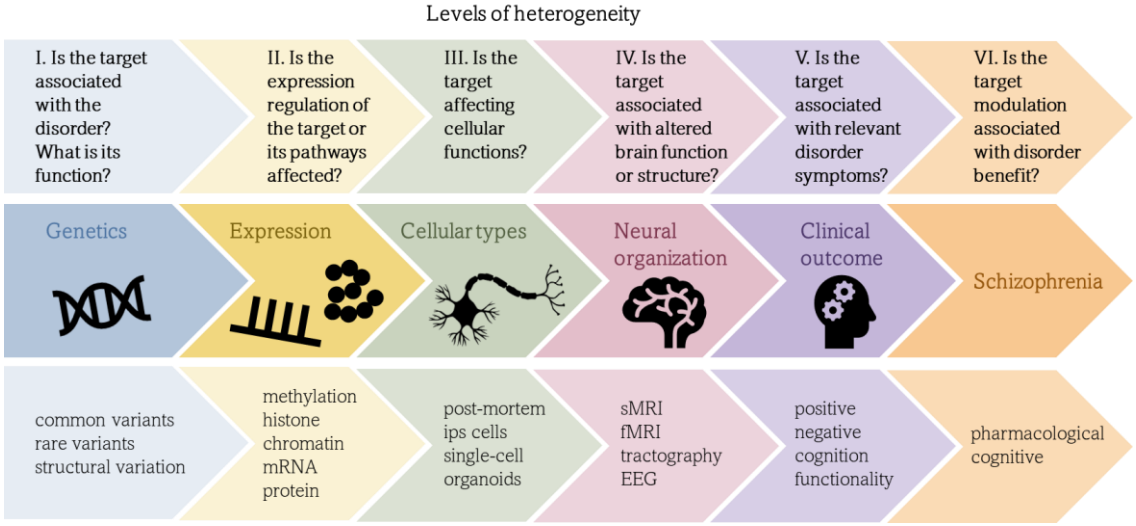


Figure 7. Illustration of multilevel heterogeneity in schizophrenia and an integrated approach to gene identification and translation. This figure presents a structured overview of schizophrenia heterogeneity, and the comprehensive approach needed for effective gene identification. The top section highlights the selection criteria proposed for gene research. The middle section illustrates various levels of molecular heterogeneity, showcasing the spectrum of effects across pathways and mechanisms. The bottom section displays examples of different research methodologies or variables to explore, each offering unique insights and contributing distinct types of information to the broader understanding.

In the path from genetic vulnerability to the complex presentation of the disorder, several questions regarding the involved genes must be answered to unravel the different levels of heterogeneity and to pinpoint potential targets. In this thesis, we have aimed to address some of the gaps in this pathway with a particular focus on *NRN1* as a model.

I. The first question to address would be whether this gene has been highlighted in previous genetic studies associated with the disorder. In the case of *NRN1*, the gene was selected based on both positional evidence and case-control genetic association studies. Several genetic linkage studies mapping schizophrenia to specific genomic locations have identified an association with the chromosome region 6p22-25 (Lindholm et al., 2001; Moises et al., 1995; Straub et al., 1995; S. Wang et al., 1995). One such study, which focused on a subtype of schizophrenia characterized by pervasive neurocognitive deficits, also pointed to the 6p24 region (Hallmayer et al., 2005). Collectively, these studies suggested *NRN1* as a positional candidate gene. Furthermore, studies on rare variants have also implicated this chromosomic region with schizophrenia, directly implicating *NRN1* (Caluseriu et al., 2006). Additionally, although *NRN1* did not appear in the most recent genome-wide association study for schizophrenia, earlier research from our group, using a case-control association study, demonstrated that *NRN1* variants are linked to an increased risk of schizophrenia spectrum disorders. This same study also linked *NRN1* genetic variability to other neuropsychiatric disorders, including bipolar disorder (Fatjó-Vilas et al., 2016). Beyond psychiatric disorders, studies on copy number variants have also documented complex alterations at chromosome 6p, which encompass candidate genes including *NRN1*, in several live-born infants presenting a range of abnormalities such as intellectual disability, growth deficiency, developmental delay, and distinct facial dysmorphisms (Linhares et al., 2015; Martinet et al., 2008; L. Zhang et al., 2023).

These varied genetic associations, spanning both rare and common variants and linking *NRN1* not only to schizophrenia and related psychiatric conditions but also to other neurodevelopmental disorders, strengthen the confidence that modulating the function of the gene could yield a clinically meaningful phenotype.

Another important consideration in identifying candidate genes as potential therapeutic targets is whether we understand the function of the gene product. Pertaining to *NRN1*, it encodes a protein that can be either GPI-anchored or secreted and it is found in both axons and dendrites of neurons (Naeve et al., 1997). Although *NRN1*, as a small GPI-anchored protein, lacks channel activity, it has been identified as part of the AMPA receptor complex, which is crucial for fast excitatory synaptic transmission in the central nervous system (Fujino et al., 2003). This complex plays a key role in synaptic plasticity processes, such as long-term potentiation and long-term depression.

Despite these insights, much remains unknown about the specific mechanisms and functions of *NRN1* within neurons. Our group is currently investigating the potential receptors for *NRN1* when it is in its soluble form. Previous studies, primarily conducted in animal models, have suggested that the insulin receptor might be a candidate, while

other research has proposed fibroblast growth factor receptor (Shimada et al., 2013; Yao et al., 2012). While these animal-based findings are important, they need to be validated in human samples. In this context, our own research, though not detailed in the present thesis, has explored post-mortem brain samples and suggests that *NRN1* may interact with *NPTN*, a type I transmembrane protein belonging to the Ig superfamily, offering new perspectives on its role in neuronal function.

An additional key issue to consider is whether the function of the gene product is relevant to the disorder. Regarding *NRN1*, as previously mentioned, animal studies have demonstrated its critical roles in brain development, including balancing neural progenitor proliferation and apoptosis, promoting neuronal migration and differentiation, supporting neurite outgrowth, and stabilizing synapses. In the adult brain, *NRN1* is essential for synaptic plasticity, a function highly relevant to treating schizophrenia, given its well-characterized expression patterns across different developmental stages and brain regions. Even more relevant for understanding the unique aspects of human brain development and disease mechanisms, research using human-derived organoids from schizophrenia patients has shown increased cell death and disrupted neuronal differentiation in ventricular zone progenitors, with *NRN1* identified as a downregulated factor in axon development, contributing to the impaired neurogenic potential of SOX2+ progenitors (Notaras et al., 2022). This downregulation highlights the critical role of *NRN1* in the pathology of schizophrenia, underscoring its importance in both brain development and disease.

II. Once a gene is identified and its function understood, the next essential step is to investigate whether regulatory mechanisms, including post-transcriptional or epigenetic modifications, alter the gene or its pathway-related products in the disorder. This involves identifying disrupted regulatory mechanisms to determine the most appropriate gene product for therapeutic intervention and assess its potential as a drug target. In terms of *NRN1*, we were not starting from scratch, as methylome-wide analyses have already linked its expression in the prefrontal cortex to schizophrenia (Pidsley et al., 2014). However, a major challenge in studying gene expression regulation in psychotic disorders is the widespread use of antipsychotics, which makes it nearly impossible to obtain post-mortem brain samples from untreated patients. Consequently, exploring how genes related to synaptic plasticity are expressed in the brain requires first determining whether they are influenced by pharmacological treatments. This limitation often compels researchers to rely on animal or cell-based studies for insight. In this sense, as previously noted, *NRN1* is sensitive to neurotherapeutic agents like electroconvulsive therapy and fluoxetine (Alme et al., 2007; Dyrvig et al., 2014; Newton et al., 2003). Therefore, understanding how such treatments impact gene expression, not only in *NRN1* but in all the associated genes, is essential for accurately interpreting its role in schizophrenia and other psychiatric disorders, highlighting the importance of complementary clinical studies to bridge these gaps.

Consequently, our post-mortem study, although focused on *NRN1*, contributes to a deeper understanding of region-specific transcriptome and methylome alterations in schizophrenia by demonstrating reduced expression of this gene in the prefrontal cortex, but not in the hippocampus, along with distinct methylation patterns that differentiate patients from controls in both regions (Alelú-Paz et al., 2016; Farsi et al., 2023; Huckins et al., 2019). Additionally, by identifying *NRN1* expression and methylation changes specifically in the prefrontal cortex of clozapine-treated patients, our study reinforces the role of epigenetic mechanisms in the effects of atypical antipsychotic treatments (Guidotti & Grayson, 2014). This is particularly significant given previous animal-based research showing that clozapine can influence chromatin remodeling and induce the demethylation of synaptic plasticity promoters, such as *Reln* and *Bdnf* (Dong et al., 2008; Guidotti et al., 2017).

Furthermore, our study, though centered on *NRN1*, presents an innovative approach for detecting methylation changes across a locus by using the sPLS-DA method. The primary advantage of sPLS-DA over traditional univariate analyses lies in its ability to manage the high correlations among adjacent CpG sites, reducing dimensionality while preserving essential information and uncovering complex multivariate relationships between methylation patterns and treatment outcomes that might otherwise remain hidden (Ruiz-Perez et al., 2020). Previous studies have used methodologies like PCA to consolidate locus methylation into latent components, as seen in research on *BDNF* methylation and brain activity (Redlich et al., 2020). However, our sPLS-DA approach uniquely enhances group separation, which increases the clinical relevance of findings. Thus, building on sPLS-DA, we adopted an integrated approach that combines a global perspective through components, a biologically meaningful direction-based analysis of CpG units within these components, and a site-specific assessment to evaluate transcriptional effects. Therefore, our results suggest that this comprehensive method offers deeper insights into the biological mechanisms underlying complex phenotypes, contributing to a more nuanced understanding of schizophrenia etiology.

By integrating these levels of analysis, we identified that global methylation changes and specific hyper- and hypomethylated positions along *NRN1* effectively distinguish clozapine-treated patients from controls. This approach enables a more detailed examination of these methylation changes in relation to other molecular variables, such as gene expression. Particularly for *NRN1*, we found that only global methylation patterns and the average of hypomethylated positions correlated with expression, suggesting a biologically specific impact. Furthermore, this method allows gene-centered analyses to be contextualized within broader methylome-wide findings, which often evaluate the combined effect of adjacent CpGs. For example, in the previously mentioned prefrontal cortex methylome-wide study of patients with schizophrenia and controls, *NRN1* methylation differences emerged only when examining the collective effect of adjacent hypomethylated CpGs (Pidsley et al., 2014).

But methylation changes at each CpG site may play distinct roles depending on its genomic context. Therefore, to understand how these methylation changes impact gene expression, it is essential to explore regulatory elements overlapping with CpG units. Using the UCSC Genome Browser (http://genome.ucsc.edu) to access ENCODE (Dunham et al., 2012) and ORegAnno data (Griffith et al., 2008), we found enhancer regions, DNase I hypersensitive sites, and histone marks such as H3K27me3, H3K4me1, and H3K36me3 within these CpG units. These findings indicate that integrating methylation data with regulatory elements offers critical insights into how epigenetic modifications may influence gene expression in schizophrenia.

Additionally, our post-mortem findings have also highlighted the importance of considering genotypic variability when investigating gene expression mechanisms, as the methylation-expression correlation of *NRN1* improved when taking genetic differences into account. To understand how genetic polymorphisms affect expression it is essential to examine their functional consequences. Using RegulomeDB (Dong & Boyle, 2019) and Haploreg (Ward & Kellis, 2016), we identified that certain *NRN1* variants linked to early-onset schizophrenia can modify histone marks and affect transcription factors binding, with some alleles notably increasing affinity for specific transcription factors involved in stress resilience through epigenetic pathways. Additionally, other variants impacting methylation-expression correlations in the prefrontal cortex also influence transcription factor binding affinities and affect enhancer and promoter functions in the brain, potentially modulating gene expression in a context-dependent manner.

Our findings highlight the altered expression of synaptic plasticity genes in schizophrenia, particularly in the prefrontal cortex, a region critical for higher-order cognitive functions and heavily implicated in the disorder. These results support the idea that targeting such genes could lead to clinically meaningful outcomes. At the same time, they underscore the complex regulation of these genes and the need for further research to better understand how genetic and epigenetic factors influence their expression, especially in the context of antipsychotic treatment.

III. Consequently, the next important question is considering how modifying the target impacts cellular function. Research, primarily from animal models, has shown that conditional knockout of the *NRN1* gene in mice delays the development and maturation of axons and dendritic arbors, as well as hinders synaptic maturation (Fujino et al., 2003). Additionally, as previously mentioned, evidence from various disease models shows that modifying *NRN1* has significant effects on neurons. For instance, silencing *Nrn1* blocks neurite outgrowth in diabetic neuropathy, while Nrn1 injections repair neurons and protect cortical cells from apoptosis in traumatic brain injury, brain infusion of recombinant Nrn1 rescued deficits in hippocampal long-term potentiation in a mice model of Alzheimer's disorder, and prevent dendritic spine degeneration and neuronal hyperexcitability in Aβ42-cultured neurons (An et al., 2014; Hurst et al., 2023; Karamoysoyli et al., 2008; Liu et al., 2018; Wan et al., 2020). Therefore, this evidence

suggests that potential drugs targeting NRN1 would have a beneficial impact on neuron homeostasis.

IV. The subsequent upper step would be considering how this gene or its pathways modify brain networks. However, prior to this thesis, the specific impact of modifying *NRN1* on brain networks had not been explored in either animal or human studies.

In this context, our neuroimaging study of early-onset individuals, a population that is challenging to study due to the low prevalence of such cases (less than 8% have an onset before age 18), provides particularly valuable insights into brain regions that may be more vulnerable to early developmental challenges (Solmi et al., 2022). Our findings suggest that early-onset schizophrenia may disrupt neural pathways involved in working memory development, leading to more significant impairments compared to adult-onset cases (Hartshorne & Germine, 2015). This is consistent with previous functional neuroimaging studies on early-onset individuals, which have repeatedly shown abnormal activation patterns in key prefrontal regions such as the ventral lateral and dorsolateral prefrontal cortex, and anterior cingulate cortex, as well as in limbic and temporal areas (Kyriakopoulos et al., 2012; Loeb et al., 2018; Pauly et al., 2008; Thormodsen et al., 2011; White et al., 2011). Additionally, our results align with a longitudinal working memory study that reported reduced recruitment in executive brain regions in early-onset adolescents, contrasting with typical developmental patterns in healthy controls (Ioakeimidis et al., 2022). These findings reinforce the idea that adolescence is a critical period for prefrontal cortex maturation and the reorganization of the working memory network (Fair et al., 2007; Finn et al., 2010; Lenroot & Giedd, 2006). As such, these insights may inform the development of targeted interventions or training programs designed specifically for early-onset individuals, focusing on the neural networks most affected by the disorder. Additionally, our specific findings on *NRN1* haplotypic variability modulating brain function and performance help bridge the gap between synaptic plasticity processes and the pathophysiological mechanisms underlying schizophrenia.

On the other hand, our findings on the epistatic interactions between *NRN1*, *BDNF*, and *CACNA1C* in relation to cortical surface area and volume highlight how these 91% heritable phenotypes, largely shaped by regulatory elements active during prenatal development, are then particularly vulnerable to the combined alterations of such genes, leading to lasting effects on the adult brain, as evidenced by structural changes (Eyler et al., 2012; Grasby et al., 2020). Additionally, although our work was conducted within a patient cohort, the brain regions modulated by epistatic effects, primarily frontal, along with some parietal and temporal regions, have previously been reported to exhibit altered cortical surface area and volume in schizophrenia patients, suggesting that epistatic networks involving synaptic plasticity genes may be among the molecular mechanisms contributing to these structural differences (Erp et al., 2018).

Although these results are associative rather than causative, they demonstrate that *NRN1* genotypic variability is linked to the modulation of brain structure and function.

From both imaging and genetic perspectives, our findings consistently highlight the prefrontal cortex as a key region implicated in schizophrenia, particularly influenced by synaptic plasticity genes. These insights underscore the crucial role of this brain region in the disorder and its susceptibility to genetic factors that regulate synaptic plasticity.

V. The following crucial stage is to explore how this gene, its pathways, and the brain networks it influences, impact clinical phenotypes. The results from studies exploring genetic associations with clinical phenotypes in schizophrenia can be framed within the concept that genetic variability can influence the phenotypic characteristics of schizophrenia in two primary ways: through susceptibility genes, which directly increase the risk of developing the disorder, and modifier genes, which impact clinical features without necessarily affecting susceptibility (Fanous & Kendler, 2005). This framework may explain the limited evidence linking *NRN1* to schizophrenia risk, as its association has primarily emerged from linkage studies consistently pointing to the chromosome 6p24-25 region, while it has not been identified in genome wide association studies (Lindholm et al., 2001). In this regard, genetic association studies suggested that *NRN1* genetic variability could be linked to specific forms of the disorder by describing its modifying effects on age at onset (Fatjó-Vilas et al., 2016). Given that early-onset forms of the disorder are particularly disabling, understanding the contribution of *NRN1* to these impairments is crucial for gaining insights into the molecular mechanisms underlying the disorder.

In this context, the work presented in this thesis helps establish *NRN1* as a key gene associated with early-onset forms of schizophrenia. Identifying the genetic factors linked to early-onset schizophrenia is clinically significant, as these cases are often associated with poorer premorbid adjustment, reduced neurocognitive performance, more severe symptoms, and a less favorable prognosis (Clemmensen et al., 2012; Larsen et al., 2004; Rajji et al., 2009; Rapoport & Gogtay, 2011). Understanding this phenotype is, therefore, essential for developing effective early intervention and prevention strategies. Additionally, early-onset schizophrenia holds theoretical significance, representing an extreme phenotype with a higher genetic load, which may provide deeper insights into the biology of the disorder and offer a stronger framework for identifying relevant genetic variants (Zhan et al., 2023). Despite the advantages of studying early onset, there have only been three genome-wide association studies focused on age at onset, and they have shown limited overlap with those targeting general schizophrenia risk, underscoring the need for further research (Bergen et al., 2014; Guo et al., 2021; Wang et al., 2011; Woolston et al., 2017). Thus, our work addresses this gap in research on age at onset and holds potential clinical implications for improving disease prediction and advancing early interventions.

Additionally, many of the aforementioned animal studies have demonstrated that the neuronal changes induce by modifying Nrn1 expression are linked to behavioral improvements, including improved neurological scores after traumatic brain injury,

recovery of spatial learning and memory after ischemia-reperfusion injury, and reduced cognitive impairments in Alzheimer's models (An et al., 2014; Liu et al., 2018; Wan et al., 2020). Therefore, beyond age at onset, *NRN1* could be modulating other phenotypes, such as symptomatology. However, exploring specific symptoms, such as positive and negative symptoms or functional impairments, is challenging with animal models, making human studies essential.

In this context, the work presented in this thesis suggests that *NRN1* acts as a modifier gene, influencing general psychopathology and functioning in combination with other pathway-related genes, such as *BDNF* and *CACNA1C*. More importantly, this research provides a valuable framework for prioritizing brain regions implicated in schizophrenia by examining the mediation effects of significant brain clusters on symptomatology. Specifically, the finding that *NRN1* x *BDNF* epistasis affects brain volume, which fully mediates its impact on symptomatology, reinforces the idea that genetic effects on clinical phenotypes are mediated by specific brain regions and that there is genetic overlap between brain structure and clinical symptoms (Sudre et al., 2020). This aligns with previous studies suggesting that genes highly expressed in brain regions with structural differences in schizophrenia are also linked to symptomatology (Ji et al., 2021; Legge, Cardno, et al., 2021; Sengupta et al., 2017).

Overall, our results demonstrate that examining the effects of candidate synaptic plasticity genes essential to schizophrenia, even when not directly associated with the disorder in large genome-wide studies but broadly linked to neurodevelopmental processes, is an effective approach for identifying genetic factors relevant to different patient groups, thereby helping to reduce variability within the disorder.

VI. Finally, having described that a gene modifies all these molecular levels, the next point is how this gene or its pathways modulate response to treatment. In this regard, prior to this thesis, although several animal-based studies suggested that *Nrn1* brain expression could be modulated by neurotherapeutic agents, the putative role of *NRN1* as biomarker for different treatments had not been explored in human studies.

In this context, our exploration of *NRN1* peripheral methylation suggests that this mechanism is dynamic and responsive to cognitive remediation therapy, consistent with previous research on the responsiveness of methylation to behavioral stimuli (Levenson et al., 2006; Miller & Sweatt, 2007). Additionally, our findings also indicate that while plastic changes in methylation serve as promising biomarkers for cognitive therapies, the genetic variability of the individual also plays a significant role in determining their response. However, it is important to note that peripheral methylation levels may not directly reflect central nervous system methylation or the mechanisms through which the therapy exerts its effects, although some studies have shown promise by revealing significant correlations in DNA methylation between blood and brain tissue (Davies et al., 2012; Walton et al., 2016). In this regard, previous research investigating the peripheral methylation of another synaptic plasticity gene, *RELN*, found changes in response to the

same therapy. These methylation levels were significantly associated with improvements in global brain network efficiency and cognitive performance, suggesting that the peripheral changes observed may have a corresponding correlate in the brain (Ho et al., 2020). These results, along with our findings, underscore the value of investigating blood methylation for biomarker identification, beyond its tissue correlations, particularly given the accessibility of the tissue and its reversible nature, which enables the examination of therapy-induced changes potentially leading to enhanced patient care (Goud Alladi et al., 2018).

Furthermore, this article highlights the clinical relevance and robustness of the sPLS-DA method for integrating complex biological data with clinical information, enabling the identification of specific biological markers that strongly correlate with certain clinical subtypes. Through the example of *NRN1*, this article offers a valuable methodological perspective that could enhance diagnostic accuracy and support the development of personalized treatment strategies, encouraging further research in this area.

But the remaining point is how we can translate this into pharmacological intervention. In this sense, there remains a significant gap in the literature regarding drug development targeting *NRN1*. In contrast to other candidate genes, such as *CACNA1C*, where calcium channel blockers targeting CaV1.2 are already widely prescribed for cardiovascular conditions and have demonstrated brain penetration and safety, we currently lack comparable information on potential drugs targeting *NRN1*. This presents an important area for future research and development.

However, both the existing evidence and the findings presented in this thesis highlight the potential of *NRN1* as a promising therapeutic target, encouraging further research into the development of potential drugs targeting this gene.

Future perspectives

Although the specific methodological details of the studies included in this thesis have been thoroughly outlined in each publication, there are several important caveats that warrant further discussion and may guide future research.

The main limitation across all the presented studies is the sample size and lack of replication samples. However, it is worth noting that, aside from large-scale studies led by international consortia, most research on modifier genes in clinical or neuroimaging phenotypes, as well as studies examining gene expression in post-mortem brain samples, typically use sample sizes comparable to ours. This limitation prevents us from examining sex-specific effects of *NRN1* at any of the studied levels, which is significant given emerging evidence that genetic risk may differ by sex (Mas-Bermejo et al., 2025).

All the variants in this study are polymorphic, so while our findings are biologically plausible, they should be interpreted with caution due to the small effect sizes of the

genetic variants on the phenotypes analyzed. In this sense, although our research highlights the role of *NRN1* in schizophrenia-related traits, it must be understood within the larger context of the complex polygenic nature of the disorder. Additionally, since rare variants also contribute to the genetic risk of schizophrenia, future studies examining the combined influence of both common and low-frequency variants in the *NRN1* gene could offer valuable insights (Singh et al., 2022).

Furthermore, also linked to the polygenic nature of the disorder, variations in a single gene are unlikely to fully explain the physiological basis of schizophrenia-related phenotypes. Thus, while our study emphasizes the role of *NRN1* in modulating these traits, the findings should be considered within the broader context of the complex genetic architecture of schizophrenia. Research consistently demonstrates that genes associated with complex traits function within networks of interacting genes and molecular pathways. To address this, we have explored the epistatic interactions between *NRN1* and its potential molecular interactors, but statistical epistasis does not necessarily imply a direct impact on gene expression. To overcome this issue, an alternative approach involves, for example, analyzing gene co-expression patterns derived from post-mortem brain samples and translating these into a polygenic co-expression index in independent samples to explore their association with other phenotypes. Using this strategy, the polygenic co-expression index for the *DRD2* gene, which predicts brain-specific expression of a network of *DRD2* co-expressed genes, has been linked to antipsychotic response, brain function during working memory in schizophrenia, and modulation of prefrontal cortex activity following D2 receptor stimulation (D'Ambrosio et al., 2022). Therefore, further studies are necessary to validate the statistical and biological impact of *NRN1* interactions on brain structure and symptoms.

A significant limitation in studying *NRN1* methylation and expression patterns is the cross-sectional nature of our research, which focuses exclusively on chronic patients. Employing a longitudinal approach or comparing first-episode, chronic, and high-risk individuals could help differentiate whether these methylation patterns are state markers, tied to the disorder phase, or trait markers, indicative of persistent features. However, since longitudinal studies cannot be conducted on post-mortem tissue, these analyses would require blood samples, which may not accurately capture brain-specific methylation changes. Additionally, interpreting the brain methylation-expression patterns in clozapine-treated patients presents complexities due to the bidirectional influence of antipsychotic treatment: antipsychotics can alter both methylation and gene expression, yet an individual's pre-treatment methylation profile can also affect how the drug functions. Without access to samples from never-treated patients, it remains difficult to separate the effects of the disorder from those of the medication, but obtaining such post-mortem samples is quite challenging. Adding to these limitations, our study concentrated only on methylation and did not investigate other epigenetic factors, such as histone deacetylation and its impact on chromatin accessibility, both of which could play crucial roles in regulating *NRN1* expression. Moreover, we did not examine *NRN1* protein levels,

even though previous research suggests that changes in the synaptic proteome are not always reflected in transcriptome data (Aryal et al., 2023). Together, these factors highlight areas for future research to provide a more comprehensive view of the role of *NRN1* in schizophrenia.

While the effects of *NRN1* are widely studied using animal and cell-based studies, the difficulty in accessing human brain tissue that sustains all these alterations has largely hampered the advances. Notably, new methodologies are opening new venues in the field. For instance, induced neural pluripotent stem cells and brain organoids represent a step forward in generating models that are closer to the biological reality and can be used to recapitulate the neurodevelopmental process offering an unprecedented vision on human-specific neurodevelopmental signatures. In this sense, the effects of *NRN1* are poorly explored, and just one study has reported *NRN1* as a downregulated factor in axon development, contributing to the impaired neurogenic potential of SOX2+ progenitors (Notaras et al., 2022).

Additionally, all of our research is performed on bulk tissues, but the application of single-cell techniques in iPSCs, brain organoids, or even post-mortem tissue, to study risk factors impacting synaptic plasticity and neurodevelopment, such as *NRN1*, could yield valuable insights into their contributions to neuropsychiatric disorders. These methods enable analysis of transcriptional changes and epigenetic modifications at a single-cell level, uncovering how genes related to brain disorders are regulated across diverse cell types and developmental stages. This is crucial in neurodevelopmental disorders, as it allows for a detailed understanding of the cellular complexity of the bran and helps pinpoint critical periods of vulnerability (Khodosevich & Sellgren, 2023; Nani et al., 2024; Wang et al., 2023). By bridging in vitro findings with in vivo relevance, single-cell techniques enhance our understanding of disease mechanisms and can inform targeted therapies. Moreover, there is growing interest in extending these techniques to peripheral tissues to explore the role of risk factors, like *NRN1*, that have significant functions in the immune system beyond the central nervous system, and to identify biomarkers across different physiological systems. (Böttcher et al., 2019; Gonzalez-Figueroa et al., 2021). In this context, we are currently using single-cell mass cytometry (CyTOF) on blood samples from individuals experiencing a first episode of psychosis, in a collaborative project originating from my PhD research stay. This technique labels immune cells with metal-tagged antibodies to detect multiple markers simultaneously, making it especially effective for profiling immune cell populations (Fernández-Zapata et al., 2020). Given the complex biological changes in early psychosis, including immune system alterations, CyTOF can identify rare immune cell subsets, cytokine profiles, and unique activation states that may be linked to the onset of psychosis.

Finally, we did not explore the diagnostic specificity of our findings, as our research focused exclusively on *NRN1* in schizophrenia. Investigating these effects in other psychotic disorders, such as bipolar disorder or autism, would be valuable not only

because of the considerable genetic overlap among these conditions but also due to the link between rare *NRN1* variants and a broad range of neurodevelopmental disorders (Linhares et al., 2015; Martinet et al., 2008; L. Zhang et al., 2023). Therefore, examining the role of *NRN1* across different diagnostic categories would improve our understanding of neurodevelopment and psychopathology.

Similarly, we did not investigate the effect of *NRN1* across different treatments, as both the study on methylation and expression correlations in the brains of schizophrenia patients and the study on peripheral methylation changes in response to cognitive remediation therapy were conducted specifically in patients treated with clozapine. Since these individuals represent a distinct subtype of the disorder with unique biological characteristics, the role of *NRN1* in response to other treatments remains an open question.

Final remarks

As a final remark, I would like to retrieve the importance of multilevel research as a potential framework to understand the role of specific candidate genes in schizophrenia. As outlined in the dissertation, human adaptive neurobiological function requires a precise neurodevelopmental plan with unique characteristics. This process depends on carefully orchestrated, spatiotemporal regulation of the transcriptome, which varies widely across individuals and shapes cognitive abilities, behavior, and personality traits. Synaptic plasticity, a defining feature essential both for brain development during maturation and for functional adaptability in adulthood, enables neurons to adjust their connections in response to external cues, refining synapses and forming neural circuits. Thus, disruptions in this mechanism, along with other regulatory processes, can lead to neuronal dysfunction and impaired neurotransmission, ultimately laying the groundwork for neurodevelopmental psychiatric disorders such as schizophrenia. Therefore, understanding how synaptic plasticity genes contribute to schizophrenia is crucial not only for deepening our knowledge of its etiological factors but also for developing more effective therapeutic strategies.

The results presented in this thesis illustrate how tracing the pathway from genetic risk to clinical presentation, specifically through a focus on *NRN1* and human association studies, can provide meaningful insights into the disorder. Using a multilevel strategy, we linked *NRN1* molecular diversity with age at onset, clinical and neuroimaging traits, examined its interactions with related genes and their impact on these traits, analyzed its expression and methylation patterns in postmortem brain samples from schizophrenia patients, and highlighted the clinical relevance of our findings by studying methylation changes in response to cognitive therapy. Furthermore, this thesis highlights, through the example of *NRN1*, that following this approach can identify existing knowledge gaps and suggest directions for future studies using advanced techniques and diverse methodologies, including animal models, preclinical research, and expanded analyses of human samples.

Overall, this thesis not only highlights the value of *NRN1* as a candidate gene for further research but also offers a methodological blueprint for investigating other synaptic plasticity genes in psychiatric disorders, thus contributing to the broader effort to unravel the complex pathways from genetic risk to clinical presentation.

7. Conclusions

The specific conclusions derived from the present thesis are:

I. Our data sheds light on the role of *NRN1* as a key gene in modulating aspects of schizophrenia presentation, including clinical manifestations, brain function during tasks involving attention and working memory, and structural brain phenotypes.

I.I. We describe specific genetic variability in the *NRN1* gene associated with the risk for early-onset schizophrenia-spectrum disorders. Additionally, early-onset cases carrying such genetic variants exhibited altered activity in the dorsolateral prefrontal cortex activity during a working memory task.

I.II. We showed that various epistatic interactions involving different SNPs of *NRN1* and *BDNF*-rs6265 or *CACNA1C*-rs1006737 significantly modulate clinical severity and neuroanatomical features in schizophrenia patients. Furthermore, we showed that the interaction between *NRN1* and *BDNF* in the left lateral orbitofrontal cortex volume fully mediates the impact on general psychopathology.

II. Our findings offer a more profound understanding of the clinical heterogeneity seen among patients, revealing through *NRN1* that expression differences between patients and controls are not uniformly distributed across the brain but exhibit region-specific patterns, which are influenced by both genetic and epigenetic factors.

II. We detected lower *NRN1* expression and methylation differences in the prefrontal cortex of clozapine-treated schizophrenia patients compared to both antipsychotic-free patients and controls, with these methylation differences correlating with *NRN1* expression and influenced by specific genetic variants along the locus.

III. Our results emphasize the importance of both epigenetic and genetic variability at *NRN1* as key factors that may serve as potential biomarkers for predicting the diverse responses to cognitive therapies.

III. We observed *NRN1* peripheral methylation changes in response to cognitive remediation therapy, indicating its responsiveness to the treatment. Additionally, these changes were linked to cognitive improvement, with global and increased methylation differences distinguishing responders from non-responders across various cognitive domains, an effect further influenced by specific genetic variants along the locus in the case of speed processing.

Our evidence, while focused on *NRN1*, reveals that synaptic plasticity genes play a crucial and multifaceted role in shaping both the cognitive and anatomical characteristics of schizophrenia, providing valuable insights into the disorder's underlying mechanisms. Moreover, they demonstrate that the complex interaction between genetic variability and epigenetic mechanisms likely drives region-specific brain expression alterations, which may explain the diverse clinical presentations observed among patients. In addition, our results highlight the link between individual genetic differences and peripheral epigenetic

profiles and their influence on therapeutic outcomes, underscoring the potential for more personalized treatment approaches. Collectively, this emphasizes the need for integrative methods to understand the role of synaptic plasticity genes, particularly *NRN1,* as pivotal factors in advancing our understanding of schizophrenia and enhancing patient care.

8. References

Aberg, K. A., McClay, J. L., Nerella, S., et al. (2014). Methylome-Wide Association Study of Schizophrenia: Identifying Blood Biomarker Signatures of Environmental Insults. *JAMA Psychiatry*, *71*(3), 255–264. https://doi.org/10.1001/jamapsychiatry.2013.3730

Aganova, É. A., & Uranova, N. A. (1992). Morphometric analysis of synaptic contacts in the anterior limbic cortex in the endogenous psychoses. *Neuroscience and Behavioral Physiology*, *22*(1), 59–65. https://doi.org/10.1007/BF01186670

Ahmed, A. O., Strauss, G. P., Buchanan, R. W., et al. (2018). Schizophrenia heterogeneity revisited: Clinical, cognitive, and psychosocial correlates of statistically-derived negative symptoms subgroups. *Journal of Psychiatric Research*, *97*, 8–15. https://doi.org/10.1016/j.jpsychires.2017.11.004

Ahn, K., An, S. S., Shugart, Y. Y., et al. (2016). Common polygenic variation and risk for childhood-onset schizophrenia. *Molecular Psychiatry*, *21*(1), 94–96. https://doi.org/10.1038/mp.2014.158

Alelú-Paz, R., Carmona, F. J., Sanchez-Mut, J. V., et al. (2016). Epigenetics in Schizophrenia: A Pilot Study of Global DNA Methylation in Different Brain Regions Associated with Higher Cognitive Functions. *Frontiers in Psychology*, *7*. https://doi.org/10.3389/fpsyg.2016.01496

Alfadil, E., & Bradke, F. (2023). Moving through the crowd. Where are we at understanding physiological axon growth? *Seminars in Cell & Developmental Biology*, *140*, 63–71. https://doi.org/10.1016/j.semcdb.2022.07.001

Alfimova, M. V., Nikitina, S. G., Lezheiko, T. V., et al. (2023). Impact on the Risk and Severity of Childhood Onset Schizophrenia of Schizophrenia Risk Genetic Variants at the DRD2 and ZNF804A Loci. *Child Psychiatry & Human Development*, *54*(1), 241–247. https://doi.org/10.1007/s10578-021-01245-z

Allen, N. C., Bagade, S., McQueen, M. B., et al. (2008). Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: The SzGene database. *Nature Genetics*, *40*(7), 827–834. https://doi.org/10.1038/ng.171

Alme, M. N., Wibrand, K., Dagestad, G., et al. (2007). Chronic Fluoxetine Treatment Induces Brain Region-Specific Upregulation of Genes Associated with BDNF-Induced Long-Term Potentiation. *Neural Plasticity*, *2007*, 26496. https://doi.org/10.1155/2007/26496

Amoretti, S., Rabelo-da-Ponte, F. D., Garriga, M., et al. (2022). Obstetric complications and cognition in schizophrenia: A systematic review and meta-analysis. *Psychological Medicine*, *52*(14), 2874–2884. https://doi.org/10.1017/S0033291722002409

An, K., Jung, J. H., Jeong, A. Y., et al. (2014). Neuritin can normalize neural deficits of Alzheimer's disease. *Cell Death & Disease*, *5*(11), e1523–e1523. https://doi.org/10.1038/cddis.2014.478

Andreasen, N. C., Arndt, S., Alliger, R., et al. (1995). Symptoms of Schizophrenia: Methods, Meanings, and Mechanisms. *Archives of General Psychiatry*, *52*(5), 341–351. https://doi.org/10.1001/archpsyc.1995.03950170015003

Andriopoulos, I., Ellul, J., Skokou, M., et al. (2011). Suicidality in the 'prodromal' phase of schizophrenia. *Comprehensive Psychiatry*, *52*(5), 479–485. https://doi.org/10.1016/j.comppsych.2010.10.011

Arnedo, J., Svrakic, D. M., del Val, C., et al. (2015). Uncovering the Hidden Risk Architecture of the Schizophrenias: Confirmation in Three Independent Genome-Wide Association Studies. *American Journal of Psychiatry*, *172*(2), 139–153. https://doi.org/10.1176/appi.ajp.2014.14040435

Aryal, S., Bonanno, K., Song, B., et al. (2023). Deep proteomics identifies shared molecular pathway alterations in synapses of patients with schizophrenia and bipolar disorder and mouse model. *Cell Reports*, *42*(5), 112497. https://doi.org/10.1016/j.celrep.2023.112497

Auton, A., Abecasis, G. R., Altshuler, D. M., et al. (2015). A global reference for human genetic variation. *Nature*, *526*(7571), 68–74. https://doi.org/10.1038/nature15393

Bachmann, S., & Schröder, J. (2018). Neurological Soft Signs in Schizophrenia: An Update on the State- versus Trait-Perspective. *Frontiers in Psychiatry*, *8*. https://doi.org/10.3389/fpsyt.2017.00272

Bachtiar, M., Jin, Y., Wang, J., et al. (2019). Architecture of population-differentiated polymorphisms in the human genome. *PLoS ONE*, *14*(10), e0224089. https://doi.org/10.1371/journal.pone.0224089

Bacon, E. R., & Brinton, R. D. (2021). Epigenetics of the developing and aging brain: Mechanisms that regulate onset and outcomes of brain reorganization. *Neuroscience & Biobehavioral Reviews*, *125*, 503–516. https://doi.org/10.1016/j.neubiorev.2021.02.040

Barr, P. B., Bigdeli, T. B., & Meyers, J. L. (2022). Prevalence, Comorbidity, and Sociodemographic Correlates of Psychiatric Diagnoses Reported in the All of Us Research Program. *JAMA Psychiatry*, *79*(6), 622–628. https://doi.org/10.1001/jamapsychiatry.2022.0685

Bassi, M. S., Iezzi, E., Gilio, L., et al. (2019). Synaptic Plasticity Shapes Brain Connectivity: Implications for Network Topology. *International Journal of Molecular Sciences*, *20*(24), 6193. https://doi.org/10.3390/ijms20246193

Baykal, S., Bozkurt, A., Çobanoğlu Osmanlı, C., et al. (2024). A comparison of clinical characteristics and course predictors in early- and childhood-onset schizophrenia. *Early Intervention in Psychiatry*. https://doi.org/10.1111/eip.13594

Beck, K., Hindley, G., Borgan, F., et al. (2020). Association of Ketamine With Psychiatric Symptoms and Implications for Its Therapeutic Use and for Understanding Schizophrenia: A Systematic Review and Meta-analysis. *JAMA Network Open*, *3*(5), e204693. https://doi.org/10.1001/jamanetworkopen.2020.4693

Belbasis, L., Köhler, C. A., Stefanis, N., et al. (2018). Risk factors and peripheral biomarkers for schizophrenia spectrum disorders: An umbrella review of meta-analyses. *Acta Psychiatrica Scandinavica*, *137*(2), 88–97. https://doi.org/10.1111/acps.12847

Benca, C. E., Derringer, J. L., Corley, R. P., et al. (2016). Predicting Cognitive Executive Functioning with Polygenic Risk Scores for Psychiatric Disorders. *Behavior Genetics*, *47*(1), 11. https://doi.org/10.1007/s10519-016-9814-2

Berdenis van Berlekom, A., Muflihah, C. H., Snijders, G. J. L. J., et al. (2020). Synapse Pathology in Schizophrenia: A Meta-analysis of Postsynaptic Elements in Postmortem Brain Studies. *Schizophrenia Bulletin*, *46*(2), 374–386. https://doi.org/10.1093/schbul/sbz060

Berendsen, S., Berendse, S., Torren, J. van der, et al. (2024). Cognitive behavioural therapy for the treatment of schizophrenia spectrum disorders: An umbrella review of meta-analyses of randomised controlled trials. *eClinicalMedicine*, *67*. https://doi.org/10.1016/j.eclinm.2023.102392

Bergen, S. E., O'Dushlaine, C. T., Lee, P. H., et al. (2014). Genetic modifiers and subtypes in schizophrenia: Investigations of age at onset, severity, sex and family history. *Schizophrenia Research*, *154*(1), 48–53. https://doi.org/10.1016/j.schres.2014.01.030

Betizeau, M., Cortay, V., Patti, D., et al. (2013). Precursor Diversity and Complexity of Lineage Relationships in the Outer Subventricular Zone of the Primate. *Neuron*, *80*(2), 442–457. https://doi.org/10.1016/j.neuron.2013.09.032

Blokland, G. A. M., McMahon, K. L., Thompson, P. M., et al. (2011). Heritability of Working Memory Brain Activation. *The Journal of Neuroscience*, *31*(30), 10882. https://doi.org/10.1523/JNEUROSCI.5334-10.2011

Bosqui, T. J., Hoy, K., & Shannon, C. (2014). A systematic review and meta-analysis of the ethnic density effect in psychotic disorders. *Social Psychiatry and Psychiatric Epidemiology*, *49*(4), 519–529. https://doi.org/10.1007/s00127-013-0773-0

Böttcher, C., Fernández-Zapata, C., Schlickeiser, S., et al. (2019). Multi-parameter immune profiling of peripheral blood mononuclear cells by multiplexed single-cell mass cytometry in patients with early multiple sclerosis. *Scientific Reports*, *9*, 19471. https://doi.org/10.1038/s41598-019-55852-x

Bourque, F., Ven, E. van der, & Malla, A. (2011). A meta-analysis of the risk for psychotic disorders among first- and second-generation immigrants. *Psychological Medicine*, *41*(5), 897–910. https://doi.org/10.1017/S0033291710001406

Bowen, E. F. W., Burgess, J. L., Granger, R., et al. (2019). DLPFC transcriptome defines two molecular subtypes of schizophrenia. *Translational Psychiatry*, *9*(1), 1–10. https://doi.org/10.1038/s41398-019-0472-z

Brown, T. A. (2002). The Human Genome. In *Genomes. 2nd edition*. Wiley-Liss. https://www.ncbi.nlm.nih.gov/books/NBK21134/

Brugger, S. P., & Howes, O. D. (2017). Heterogeneity and Homogeneity of Regional Brain Structure in Schizophrenia. *JAMA Psychiatry*, *74*(11), 1104–1111. https://doi.org/10.1001/jamapsychiatry.2017.2663

Burns, J. K., Tomita, A., & Kapadia, A. S. (2014). Income inequality and schizophrenia: Increased schizophrenia incidence in countries with high levels of income inequality. *The International Journal of Social Psychiatry*, *60*(2), 185–196. https://doi.org/10.1177/0020764013481426

Byrne, M., Agerbo, E., Ewald, H., et al. (2003). Parental Age and Risk of Schizophrenia: A Case-control Study. *Archives of General Psychiatry*, *60*(7), 673–678. https://doi.org/10.1001/archpsyc.60.7.673

Caluseriu, O., Mirza, G., Ragoussis, J., et al. (2006). Schizophrenia in an adult with 6p25 deletion syndrome. *American Journal of Medical Genetics Part A*, *140A*(11), 1208–1213. https://doi.org/10.1002/ajmg.a.31222

Cantallops, I., Haas, K., & Cline, H. T. (2000). Postsynaptic CPG15 promotes synaptic maturation and presynaptic axon arbor elaboration in vivo. *Nature Neuroscience*, *3*(10), 1004–1011. https://doi.org/10.1038/79823

Cappelletti, G., Galbiati, M., Ronchi, C., et al. (2007). Neuritin (cpg15) enhances the differentiating effect of NGF on neuronal PC12 cells. *Journal of Neuroscience Research*, *85*(12), 2702–2713. https://doi.org/10.1002/jnr.21235

Carbon, M., & Correll, C. U. (2014). Thinking and acting beyond the positive: The role of the cognitive and negative symptoms in schizophrenia. *CNS Spectrums*, *19 Suppl 1*, 38–52; quiz 35–37, 53. https://doi.org/10.1017/S1092852914000601

Cardno, A. G., & Gottesman, I. I. (2000). Twin studies of schizophrenia: From bow-and-arrow concordances to Star Wars Mx and functional genomics. *American Journal of Medical Genetics*, *97*(1), 12–17. https://doi.org/10.1002/(SICI)1096-8628(200021)97:1<12::AID-AJMG3>3.0.CO;2-U

Cattarinussi, G., Delvecchio, G., Sambataro, F., et al. (2022). The effect of polygenic risk scores for major depressive disorder, bipolar disorder and schizophrenia on morphological brain measures: A systematic review of the evidence. *Journal of Affective Disorders*, *310*, 213–222. https://doi.org/10.1016/j.jad.2022.05.007

Chan, R. F., Shabalin, A. A., Montano, C., et al. (2020). Independent Methylome-Wide Association Studies of Schizophrenia Detect Consistent Case–Control Differences. *Schizophrenia Bulletin*, *46*(2), 319–327. https://doi.org/10.1093/schbul/sbz056

Chandler, D., Dragović, M., Cooper, M., et al. (2010). Impact of Neuritin 1 (NRN1) polymorphisms on fluid intelligence in schizophrenia. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *153B*(2), 428–437. https://doi.org/10.1002/ajmg.b.30996

Charlson, F. J., Ferrari, A. J., Santomauro, D. F., et al. (2018). Global Epidemiology and Burden of Schizophrenia: Findings From the Global Burden of Disease Study 2016. *Schizophrenia Bulletin*, *44*(6), 1195–1203. https://doi.org/10.1093/schbul/sby058

Chen, C., Zhang, C., Cheng, L., et al. (2014). Correlation between DNA methylation and gene expression in the brains of patients with bipolar disorder and schizophrenia. *Bipolar Disorders*, *16*(8), 790–799. https://doi.org/10.1111/bdi.12255

Chen X., Tia C., Chen J., et al. (2011). The expression of Neurttin protein in the visual cortex of monocular form deprived adult rats. *Zhonghua Yan Ke Za Zhi*, *47*(09), Article 09. https://doi.org/10.3760/cma.j.issn.0412-4081.2011.09.014

Choi, S. W., Mak, T. S.-H., & O'Reilly, P. F. (2020). Tutorial: A guide to performing polygenic risk score analyses. *Nature Protocols*, *15*(9), 2759–2772. https://doi.org/10.1038/s41596-020-0353-1

Ciuculete, D. M., Boström, A. E., Voisin, S., et al. (2017). A methylome-wide mQTL analysis reveals associations of methylation sites with GAD1 and HDAC3 SNPs and a general psychiatric risk score. *Translational Psychiatry*, *7*(1), e1002–e1002. https://doi.org/10.1038/tp.2016.275

Clark, L. A., Cuthbert, B., Lewis-Fernández, R., et al. (2017). Three Approaches to Understanding and Classifying Mental Disorder: ICD-11, DSM-5, and the National Institute of Mental Health's Research Domain Criteria (RDoC). *Psychological Science in the Public Interest*, *18*(2), 72–145. https://doi.org/10.1177/1529100617727266

Clemmensen, L., Vernal, D. L., & Steinhausen, H.-C. (2012). A systematic review of the long-term outcome of early onset schizophrenia. *BMC Psychiatry*, *12*(1), 150. https://doi.org/10.1186/1471-244X-12-150

Coldwell, C. M., & Bender, W. S. (2007). The Effectiveness of Assertive Community Treatment for Homeless Populations With Severe Mental Illness: A Meta-Analysis. *American Journal of Psychiatry*, *164*(3), 393–399. https://doi.org/10.1176/ajp.2007.164.3.393

Collado-Torres, L., Burke, E. E., Peterson, A., et al. (2019). Regional Heterogeneity in Gene Expression, Regulation, and Coherence in the Frontal Cortex and Hippocampus across Development and Schizophrenia. *Neuron*, *103*(2), 203-216.e8. https://doi.org/10.1016/j.neuron.2019.05.013

Collins, A. L., Kim, Y., Sklar, P., et al. (2012). Hypothesis-driven candidate genes for schizophrenia compared to genome-wide association results. *Psychological Medicine*, *42*(3), 607–616. https://doi.org/10.1017/S0033291711001607

Correll, C. U., Solmi, M., Croatto, G., et al. (2022). Mortality in people with schizophrenia: A systematic review and meta-analysis of relative risk and aggravating or attenuating factors. *World Psychiatry*, *21*(2), 248–271. https://doi.org/10.1002/wps.20994

Corriveau, R. A., Shatz, C. J., & Nedivi, E. (1999). Dynamic Regulation of cpg15 during Activity-Dependent Synaptic Development in the Mammalian Visual System. *The Journal of Neuroscience*, *19*(18), 7999–8008. https://doi.org/10.1523/JNEUROSCI.19-18-07999.1999

Crow, T. J., & Done, D. J. (1986). Age of onset of schizophrenia in siblings: A test of the contagion hypothesis. *Psychiatry Research*, *18*(2), 107–117. https://doi.org/10.1016/0165-1781(86)90023-5

Cummings, J. A., Mulkey, R. M., Nicoll, R. A., et al. (1996). Ca2+ signaling requirements for long-term depression in the hippocampus. *Neuron*, *16*(4), 825–833. https://doi.org/10.1016/s0896-6273(00)80102-6

Cuthbert, B. N., & Morris, S. E. (2021). Evolving Concepts of the Schizophrenia Spectrum: A Research Domain Criteria Perspective. *Frontiers in Psychiatry*, *12*. https://doi.org/10.3389/fpsyt.2021.641319

Czepielewski, L. S., Massuda, R., Goi, P., et al. (2015). Verbal episodic memory along the course of schizophrenia and bipolar disorder: A new perspective. *European Neuropsychopharmacology*, *25*(2), 169–175. https://doi.org/10.1016/j.euroneuro.2014.09.006

D'Ambrosio, E., Pergola, G., Pardiñas, A. F., et al. (2022). A polygenic score indexing a DRD2-related co-expression network is associated with striatal dopamine function. *Scientific Reports*, *12*(1), 12610. https://doi.org/10.1038/s41598-022-16442-6

Davies, M. N., Volta, M., Pidsley, R., et al. (2012). Functional annotation of the human brain methylome identifies tissue-specific epigenetic variation across brain and blood. *Genome Biology*, *13*(6), R43. https://doi.org/10.1186/gb-2012-13-6-r43

de Greef, R., Maloney, A., Olsson-Gisleskog, P., et al. (2011). Dopamine D2 occupancy as a biomarker for antipsychotics: Quantifying the relationship with efficacy and extrapyramidal symptoms. *The AAPS Journal*, *13*(1), 121–130. https://doi.org/10.1208/s12248-010-9247-4

Dehay, C., Kennedy, H., & Kosik, K. S. (2015). The outer subventricular zone and primate-specific cortical complexification. *Neuron*, *85*(4), 683–694. https://doi.org/10.1016/j.neuron.2014.12.060

Dempster, E. L., Pidsley, R., Schalkwyk, L. C., et al. (2011). Disease-associated epigenetic changes in monozygotic twins discordant for schizophrenia and bipolar disorder. *Human Molecular Genetics*, *20*(24), 4786–4796. https://doi.org/10.1093/hmg/ddr416

DeRosse, P., & Karlsgodt, K. H. (2015). Examining the Psychosis Continuum. *Current Behavioral Neuroscience Reports*, *2*(2), 80. https://doi.org/10.1007/s40473-015-0040-7

Díaz-Caneja, C. M., Pina-Camacho, L., Rodríguez-Quiroga, A., et al. (2015). Predictors of outcome in early-onset psychosis: A systematic review. *NPJ Schizophrenia*, *1*, 14005. https://doi.org/10.1038/npjschz.2014.5

Dickson, H., Laurens, K. R., Cullen, A. E., et al. (2012). Meta-analyses of cognitive and motor function in youth aged 16 years and younger who subsequently develop schizophrenia. *Psychological Medicine*, *42*(4), 743–755. https://doi.org/10.1017/S0033291711001693

Dong, D., Wang, Y., Chang, X., et al. (2018). Dysfunction of Large-Scale Brain Networks in Schizophrenia: A Meta-analysis of Resting-State Functional Connectivity. *Schizophrenia Bulletin*, *44*(1), 168–181. https://doi.org/10.1093/schbul/sbx034

Dong, E., Nelson, M., Grayson, D. R., et al. (2008). Clozapine and sulpiride but not haloperidol or olanzapine activate brain DNA demethylation. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(36), 13614. https://doi.org/10.1073/pnas.0805493105

Dong, S., & Boyle, A. P. (2019). Predicting functional variants in enhancer and promoter elements using RegulomeDB. *Human Mutation*, *40*(9), 1292–1298. https://doi.org/10.1002/humu.23791

Dunham, I., Kundaje, A., Aldred, S. F., et al. (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature*, *489*(7414), 57–74. https://doi.org/10.1038/nature11247

Dyrvig, M., Christiansen, S. H., Woldbye, D. P. D., et al. (2014). Temporal gene expression profile after acute electroconvulsive stimulation in the rat. *Gene*, *539*(1), 8–14. https://doi.org/10.1016/j.gene.2014.01.072

Eack, S. M., Hogarty, G. E., Cho, R. Y., et al. (2010). Neuroprotective Effects of Cognitive Enhancement Therapy Against Gray Matter Loss in Early Schizophrenia: Results From a 2-Year Randomized Controlled Trial. *Archives of General Psychiatry*, *67*(7), 674–682. https://doi.org/10.1001/archgenpsychiatry.2010.63

Edwards, A. C., Bigdeli, T. B., Docherty, A. R., et al. (2016). Meta-analysis of Positive and Negative Symptoms Reveals Schizophrenia Modifier Genes. *Schizophrenia Bulletin*, *42*(2), 279–287. https://doi.org/10.1093/schbul/sbv119

Egerton, A., Chaddock, C. A., Winton-Brown, T. T., et al. (2013). Presynaptic Striatal Dopamine Dysfunction in People at Ultra-high Risk for Psychosis: Findings in a Second Cohort. *Biological Psychiatry*, *74*(2), 106–112. https://doi.org/10.1016/j.biopsych.2012.11.017

El-Sayed, M., Hofman-Bang, J., & Mikkelsen, J. D. (2011). Effect of brain-derived neurotrophic factor on activity-regulated cytoskeleton-associated protein gene expression in primary frontal cortical neurons. Comparison with NMDA and AMPA. *European Journal of Pharmacology*, *660*(2), 351–357. https://doi.org/10.1016/j.ejphar.2011.03.055

Emsley, R., Rabinowitz, J., & Medori, R. (2006). Time course for antipsychotic treatment response in first-episode schizophrenia. *The American Journal of Psychiatry*, *163*(4), 743–745. https://doi.org/10.1176/ajp.2006.163.4.743

Endicott, J., Spitzer, R. L., Fleiss, J. L., et al. (1976). The Global Assessment Scale: A Procedure for Measuring Overall Severity of Psychiatric Disturbance. *Archives of General Psychiatry*, *33*(6), 766–771. https://doi.org/10.1001/archpsyc.1976.01770060086012

Erp, T. G. M. van, Walton, E., Hibar, D. P., et al. (2018). Cortical Brain Abnormalities in 4474 Individuals With Schizophrenia and 5098 Control Subjects via the Enhancing Neuro Imaging Genetics Through Meta Analysis (ENIGMA) Consortium. *Biological Psychiatry*, *84*(9), 644–654. https://doi.org/10.1016/j.biopsych.2018.04.023

Ewens, W. J., & Spielman, R. S. (1995). The transmission/disequilibrium test: History, subdivision, and admixture. *American Journal of Human Genetics*, *57*(2), 455.

Eyler, L. T., Chen, C.-H., Panizzon, M. S., et al. (2012). A Comparison of Heritability Maps of Cortical Surface Area and Thickness and the Influence of Adjustment for Whole Brain Measures: A Magnetic Resonance Imaging Twin Study. *Twin Research and Human Genetics : The Official Journal of the International Society for Twin Studies*, *15*(3), 304–314. https://doi.org/10.1017/thg.2012.3

Fair, D. A., Dosenbach, N. U. F., Church, J. A., et al. (2007). Development of distinct control networks through segregation and integration. *Proceedings of the National Academy of Sciences*, *104*(33), 13507–13512. https://doi.org/10.1073/pnas.0705843104

Fanous, A. H., & Kendler, K. S. (2005). Genetic heterogeneity, modifier genes, and quantitative phenotypes in psychiatric illness: Searching for a framework. *Molecular Psychiatry*, *10*(1), 6–13. https://doi.org/10.1038/sj.mp.4001571

Fanous, A. H., van den Oord, E. J., Riley, B. P., et al. (2005). Relationship Between a High-Risk Haplotype in the DTNBP1 (Dysbindin) Gene and Clinical Features of Schizophrenia. *American Journal of Psychiatry*, *162*(10), 1824–1832. https://doi.org/10.1176/appi.ajp.162.10.1824

Farsi, Z., Nicolella, A., Simmons, S. K., et al. (2023). Brain-region-specific changes in neurons and glia and dysregulation of dopamine signaling in Grin2a mutant mice. *Neuron*, *111*(21), 3378-3396.e9. https://doi.org/10.1016/j.neuron.2023.08.004

Fatjó-Vilas, M., Papiol, S., Estrada, G., et al. (2011). Dysbindin-1 gene contributes differentially to early- and adult-onset forms of functional psychosis. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *156*(3), 322–333. https://doi.org/10.1002/ajmg.b.31166

Fatjó-Vilas, M., Prats, C., Pomarol-Clotet, E., et al. (2016). Involvement of NRN1 gene in schizophrenia-spectrum and bipolar disorders and its impact on age at onset and cognitive functioning. *The World Journal of Biological Psychiatry*, *17*(2), 129–139. https://doi.org/10.3109/15622975.2015.1093658

Faust, T. E., Gunner, G., & Schafer, D. P. (2021). Mechanisms governing activity-dependent synaptic pruning in the developing mammalian CNS. *Nature Reviews Neuroscience*, *22*(11), 657–673. https://doi.org/10.1038/s41583-021-00507-y

Faux, C., Rakic, S., Andrews, W., et al. (2012). Neurons on the move: Migration and lamination of cortical interneurons. *Neuro-Signals*, *20*(3), 168–189. https://doi.org/10.1159/000334489

Feinberg, I. (1982). Schizophrenia: Caused by a fault in programmed synaptic elimination during adolescence? *Journal of Psychiatric Research*, *17*(4), 319–334. https://doi.org/10.1016/0022-3956(82)90038-3

Feng, J., Zhou, Y., Campbell, S. L., et al. (2010). Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. *Nature Neuroscience*, *13*(4), 423–430. https://doi.org/10.1038/nn.2514

Fernández-Zapata, C., Leman, J. K. H., Priller, J., et al. (2020). The use and limitations of single-cell mass cytometry for studying human microglia function. *Brain Pathology*, *30*(6), 1178–1191. https://doi.org/10.1111/bpa.12909

Fett, A.-K. J., Lemmers-Jansen, I. L. J., & Krabbendam, L. (2019). Psychosis and urbanicity: A review of the recent literature from epidemiology to neurourbanism. *Current Opinion in Psychiatry*, *32*(3), 232–241. https://doi.org/10.1097/YCO.0000000000000486

Finn, A. S., Sheridan, M. A., Kam, C. L. H., et al. (2010). Longitudinal Evidence for Functional Specialization of the Neural Circuit Supporting Working Memory in the Human Brain. *Journal of Neuroscience*, *30*(33), 11062–11067. https://doi.org/10.1523/JNEUROSCI.6266-09.2010

Fischl, B. (2012). FreeSurfer. *NeuroImage*, *62*(2), 774–781. https://doi.org/10.1016/j.neuroimage.2012.01.021

Fisher, H. L., Murphy, T. M., Arseneault, L., et al. (2015). Methylomic analysis of monozygotic twins discordant for childhood psychotic symptoms. *Epigenetics*, *10*(11), 1014–1023. https://doi.org/10.1080/15592294.2015.1099797

Föcking, M., Doyle, B., Munawar, N., et al. (2019). Epigenetic Factors in Schizophrenia: Mechanisms and Experimental Approaches. *Molecular Neuropsychiatry*, *5*(1), 6–12. https://doi.org/10.1159/000495063

Foley, C., Corvin, A., & Nakagome, S. (2017). Genetics of Schizophrenia: Ready to Translate? *Current Psychiatry Reports*, *19*(9), 61. https://doi.org/10.1007/s11920-017-0807-5

Fond, G., Vidal, M., Joseph, M., et al. (2023). Self-stigma in schizophrenia: A systematic review and meta-analysis of 37 studies from 25 high- and low-to-middle income countries. *Molecular Psychiatry*, *28*(5), 1920–1931. https://doi.org/10.1038/s41380-023-02003-4

Franks, A., Airoldi, E., & Slavov, N. (2017). Post-transcriptional regulation across human tissues. *PLOS Computational Biology*, *13*(5), e1005535. https://doi.org/10.1371/journal.pcbi.1005535

Frederick, D. E., & VanderWeele, T. J. (2019). Supported employment: Meta-analysis and review of randomized controlled trials of individual placement and support. *PLoS ONE*, *14*(2), e0212208. https://doi.org/10.1371/journal.pone.0212208

Fromer, M., Pocklington, A. J., Kavanagh, D. H., et al. (2014). De novo mutations in schizophrenia implicate synaptic networks. *Nature*, *506*(7487), 179–184. https://doi.org/10.1038/nature12929

Fujino, T., Lee, W.-C. A., & Nedivi, E. (2003). Regulation of cpg15 by signaling pathways that mediate synaptic plasticity. *Molecular and Cellular Neuroscience*, *24*(3), 538–554. https://doi.org/10.1016/S1044-7431(03)00230-6

Fujino, T., Leslie, J. H., Eavri, R., et al. (2011). CPG15 regulates synapse stability in the developing and adult brain. *Genes & Development*, *25*(24), 2674–2685. https://doi.org/10.1101/gad.176172.111

Fujino, T., Wu, Z., Lin, W. C., et al. (2008). Cpg15 and cpg15-2 Constitute a Family of Activity-Regulated Ligands Expressed Differentially in the Nervous System to Promote Neurite Growth and Neuronal Survival. *The Journal of Comparative Neurology*, *507*(5), 1831–1845. https://doi.org/10.1002/cne.21649

Fuller, R., Nopoulos, P., Arndt, S., et al. (2002). Longitudinal Assessment of Premorbid Cognitive Functioning in Patients With Schizophrenia Through Examination of Standardized Scholastic Test Performance. *American Journal of Psychiatry*, *159*(7), 1183–1189. https://doi.org/10.1176/appi.ajp.159.7.1183

GBD 2019 Mental Disorders Collaborators. (2022). Global, regional, and national burden of 12 mental disorders in 204 countries and territories, 1990–2019: A systematic analysis for the Global Burden of Disease Study 2019. *The Lancet Psychiatry*, *9*(2), 137–150. https://doi.org/10.1016/S2215-0366(21)00395-3

Genovese, G., Fromer, M., Stahl, E. A., et al. (2016). Increased burden of ultra-rare protein-altering variants among 4,877 individuals with schizophrenia. *Nature Neuroscience*, *19*(11), 1433–1441. https://doi.org/10.1038/nn.4402

George, M., Maheshwari, S., Chandran, S., et al. (2017). Understanding the schizophrenia prodrome. *Indian Journal of Psychiatry*, *59*(4), 505–509. https://doi.org/10.4103/psychiatry.IndianJPsychiatry_464_17

Ghadirivasfi, M., Nohesara, S., Ahmadkhaniha, H.-R., et al. (2011). Hypomethylation of the serotonin receptor type-2A Gene (HTR2A) at T102C polymorphic site in DNA derived from the saliva of patients with schizophrenia and bipolar disorder. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics*, *156B*(5), 536–545. https://doi.org/10.1002/ajmg.b.31192

Ginovart, N., & Kapur, S. (2012). Role of dopamine D(2) receptors for antipsychotic activity. *Handbook of Experimental Pharmacology*, *212*, 27–52. https://doi.org/10.1007/978-3-642-25761-2_2

Glahn, D. C., Laird, A. R., Ellison-Wright, I., et al. (2008). Meta-Analysis of Gray Matter Anomalies in Schizophrenia: Application of Anatomic Likelihood Estimation and Network Analysis. *Biological Psychiatry*, *64*(9), 774–781. https://doi.org/10.1016/j.biopsych.2008.03.031

Glahn, D. C., Nimgaonkar, V. L., Raventós, H., et al. (2019). Rediscovering the value of families for psychiatric genetics research. *Molecular Psychiatry*, *24*(4), 523–535. https://doi.org/10.1038/s41380-018-0073-x

Glahn, D. C., Winkler, A. M., Kochunov, P., et al. (2010). Genetic control over the resting brain. *Proceedings of the National Academy of Sciences*, *107*(3), 1223–1228. https://doi.org/10.1073/pnas.0909969107

Glatt, S. J., Faraone, S. V., & Tsuang, M. T. (2003). Association Between a Functional Catechol O-Methyltransferase Gene Polymorphism and Schizophrenia: Meta-Analysis of Case-Control and

Family-Based Studies. *American Journal of Psychiatry*, *160*(3), 469–476. https://doi.org/10.1176/appi.ajp.160.3.469

Gogtay, N., Giedd, J. N., Lusk, L., et al. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(21), 8174–8179. https://doi.org/10.1073/pnas.0402680101

Golembo-Smith, S., Walder, D. J., Daly, M. P., et al. (2012). The presentation of dermatoglyphic abnormalities in schizophrenia: A meta-analytic review. *Schizophrenia Research*, *142*(1), 1–11. https://doi.org/10.1016/j.schres.2012.10.002

González-Castro, T. B., Hernández-Díaz, Y., Juárez-Rojop, I. E., et al. (2016). The Role of a Catechol-O-Methyltransferase (COMT) Val158Met Genetic Polymorphism in Schizophrenia: A Systematic Review and Updated Meta-analysis on 32,816 Subjects. *Neuromolecular Medicine*, *18*(2), 216–231. https://doi.org/10.1007/s12017-016-8392-z

Gonzalez-Figueroa, P., Roco, J. A., Papa, I., et al. (2021). Follicular regulatory T cells produce neuritin to regulate B cells. *Cell*, *184*(7), 1775-1789.e19. https://doi.org/10.1016/j.cell.2021.02.027

Gooding, D. C. (2022). Brave New World: Harnessing the promise of biomarkers to help solve the epigenetic puzzle. *Schizophrenia Research*, *242*, 35–41. https://doi.org/10.1016/j.schres.2022.01.020

Goto, M., Abe, O., Hagiwara, A., et al. (2022). Advantages of Using Both Voxel- and Surface-based Morphometry in Cortical Morphology Analysis: A Review of Various Applications. *Magnetic Resonance in Medical Sciences*, *21*(1), 41. https://doi.org/10.2463/mrms.rev.2021-0096

Gottesman, I. I. (1991). *Schizophrenia Genesis: The Origins of Madness*. Freeman and Co.

Gottesman, I. I., & Bertelsen, A. (1989). Confirming unexpressed genotypes for schizophrenia. Risks in the offspring of Fischer's Danish identical and fraternal discordant twins. *Archives of General Psychiatry*, *46*(10), 867–872. https://doi.org/10.1001/archpsyc.1989.01810100009002

Goud Alladi, C., Etain, B., Bellivier, F., et al. (2018). DNA Methylation as a Biomarker of Treatment Response Variability in Serious Mental Illnesses: A Systematic Review Focused on Bipolar Disorder, Schizophrenia, and Major Depressive Disorder. *International Journal of Molecular Sciences*, *19*(10), Article 10. https://doi.org/10.3390/ijms19103026

Grasby, K. L., Jahanshad, N., Painter, J. N., et al. (2020). The genetic architecture of the human cerebral cortex. *Science*, *367*(6484), eaay6690. https://doi.org/10.1126/science.aay6690

Griffith, O. L., Montgomery, S. B., Bernier, B., et al. (2008). ORegAnno: An open-access community-driven resource for regulatory annotation. *Nucleic Acids Research*, *36*(Database issue), D107–D113. https://doi.org/10.1093/nar/gkm967

Grover, V. P., Tognarelli, J. M., Crossey, M. M., et al. (2015). Magnetic Resonance Imaging: Principles and Techniques: Lessons for Clinicians. *Journal of Clinical and Experimental Hepatology*, *5*(3), 246. https://doi.org/10.1016/j.jceh.2015.08.001

Guidotti, A., Dong, E., & Grayson, D. R. (2017). *Epigenetic Basis of Clozapine Action*.

Guidotti, A., & Grayson, D. R. (2014). DNA methylation and demethylation as targets for antipsychotic therapy. *Dialogues in Clinical Neuroscience*, *16*(3), 419–429. https://doi.org/10.31887/DCNS.2014.16.3/aguidotti

Guidotti, A., Grayson, D. R., & Caruncho, H. J. (2016). Epigenetic RELN Dysfunction in Schizophrenia and Related Neuropsychiatric Disorders. *Frontiers in Cellular Neuroscience*, *10*. https://doi.org/10.3389/fncel.2016.00089

Guo, S., Liu, J., Li, W., et al. (2021). Genome wide association study identifies four loci for early onset schizophrenia. *Translational Psychiatry*, *11*(1), 1–11. https://doi.org/10.1038/s41398-021-01360-4

Hafner, H., Maurer, K., Loffler, W., et al. (2003). Modeling the Early Course of Schizophrenia. *Schizophrenia Bulletin*, *29*(2), 325–340. https://doi.org/10.1093/oxfordjournals.schbul.a007008

Haijma, S. V., Van Haren, N., Cahn, W., et al. (2013). Brain Volumes in Schizophrenia: A Meta-Analysis in Over 18 000 Subjects. *Schizophrenia Bulletin*, *39*(5), 1129–1138. https://doi.org/10.1093/schbul/sbs118

Halder, R., Hennion, M., Vidal, R. O., et al. (2016). DNA methylation changes in plasticity genes accompany the formation and maintenance of memory. *Nature Neuroscience*, *19*(1), 102–110. https://doi.org/10.1038/nn.4194

Hallmayer, J. F., Kalaydjieva, L., Badcock, J., et al. (2005). Genetic Evidence for a Distinct Subtype of Schizophrenia Characterized by Pervasive Cognitive Deficit. *The American Journal of Human Genetics*, *77*(3), 468–476. https://doi.org/10.1086/432816

Hanamsagar, R., & Bilbo, S. D. (2017). Environment Matters: Microglia Function and Dysfunction in a Changing World. *Current Opinion in Neurobiology*, *47*, 146. https://doi.org/10.1016/j.conb.2017.10.007

Hannon, E., Dempster, E., Viana, J., et al. (2016). An integrated genetic-epigenetic analysis of schizophrenia: Evidence for co-localization of genetic associations and differential DNA methylation. *Genome Biology*, *17*(1), 176. https://doi.org/10.1186/s13059-016-1041-x

Hare, E., Glahn, D. C., Dassori, A., et al. (2010). Heritability of age of onset of psychosis in schizophrenia. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics*, *153B*(1), 298–302. https://doi.org/10.1002/ajmg.b.30959

Harper, S., Towers-Evans, H., & MacCabe, J. (2015). The aetiology of schizophrenia: What have the Swedish Medical Registers taught us? *Social Psychiatry and Psychiatric Epidemiology*, *50*(10), 1471–1479. https://doi.org/10.1007/s00127-015-1081-7

Hartshorne, J. K., & Germine, L. T. (2015). When Does Cognitive Functioning Peak? The Asynchronous Rise and Fall of Different Cognitive Abilities Across the Life Span. *Psychological Science*, *26*(4), 433–443. https://doi.org/10.1177/0956797614567339

Harwell, C., Burbach, B., Svoboda, K., et al. (2005). Regulation of cpg15 Expression during Single Whisker Experience in the Barrel Cortex of Adult Mice. *Journal of Neurobiology*, *65*(1), 85–96. https://doi.org/10.1002/neu.20176

Hatanaka, Y., & Hirata, T. (2020). How Do Cortical Excitatory Neurons Terminate Their Migration at the Right Place? Critical Roles of Environmental Elements. *Frontiers in Cell and Developmental Biology*, *8*. https://doi.org/10.3389/fcell.2020.596708

He, Q., Jantac Mam-Lam-Fook, C., Chaignaud, J., et al. (2021). Influence of polygenic risk scores for schizophrenia and resilience on the cognition of individuals at-risk for psychosis. *Translational Psychiatry*, *11*(1), 1–9. https://doi.org/10.1038/s41398-021-01624-z

Heald, A., Pendlebury, J., Anderson, S., et al. (2017). Lifestyle factors and the metabolic syndrome in Schizophrenia: A cross-sectional study. *Annals of General Psychiatry*, *16*, 12. https://doi.org/10.1186/s12991-017-0134-6

Health, S. (700, 37:21). *Does DNA Methylation Increase Gene Expression? Your Script For Good Health*. Seeking Health. https://www.seekinghealth.com/blogs/education/does-dna-methylation-increase-gene-expression-your-script-for-good-health

Henriksen, M. G., Nordgaard, J., & Jansson, L. B. (2017). Genetics of Schizophrenia: Overview of Methods, Findings and Limitations. *Frontiers in Human Neuroscience*, *11*. https://doi.org/10.3389/fnhum.2017.00322

Herculano-Houzel, S. (2009). The human brain in numbers: A linearly scaled-up primate brain. *Frontiers in Human Neuroscience*, *3*. https://doi.org/10.3389/neuro.09.031.2009

Hilker, R., Helenius, D., Fagerlund, B., et al. (2017). Is an Early Age at Illness Onset in Schizophrenia Associated With Increased Genetic Susceptibility? Analysis of Data From the Nationwide Danish Twin Register. *EBioMedicine*, *18*, 320–326. https://doi.org/10.1016/j.ebiom.2017.04.002

Hilker, R., Helenius, D., Fagerlund, B., et al. (2018). Heritability of Schizophrenia and Schizophrenia Spectrum Based on the Nationwide Danish Twin Register. *Biological Psychiatry*, *83*(6), 492–498. https://doi.org/10.1016/j.biopsych.2017.08.017

Hillman, E. M. (2014). Coupling Mechanism and Significance of the BOLD Signal: A Status Report. *Annual Review of Neuroscience*, *37*, 161. https://doi.org/10.1146/annurev-neuro-071013-014111

Hipes, C., Lucas, J., Phelan, J. C., et al. (2016). The stigma of mental illness in the labor market. *Social Science Research*, *56*, 16–25. https://doi.org/10.1016/j.ssresearch.2015.12.001

Ho, N. F., Tng, J. X. J., Wang, M., et al. (2020). Plasticity of DNA methylation, functional brain connectivity and efficiency in cognitive remediation for schizophrenia. *Journal of Psychiatric Research*, *126*, 122–133. https://doi.org/10.1016/j.jpsychires.2020.03.013

Holler, S., Köstinger, G., Martin, K. A. C., et al. (2021). Structure and function of a neocortical synapse. *Nature*, *591*(7848), 111–116. https://doi.org/10.1038/s41586-020-03134-2

Hor, K., & Taylor, M. (2010). Suicide and schizophrenia: A systematic review of rates and risk factors. *Journal of Psychopharmacology (Oxford, England)*, *24*(4_supplement), 81–90. https://doi.org/10.1177/1359786810385490

Howes, O. D., McCutcheon, R., Agid, O., et al. (2016). Treatment resistant schizophrenia: Treatment Response and Resistance in Psychosis (TRRIP) working group consensus guidelines on diagnosis and terminology. *The American Journal of Psychiatry*, *174*(3), 216. https://doi.org/10.1176/appi.ajp.2016.16050503

Howes, O. D., & Onwordi, E. C. (2023). The synaptic hypothesis of schizophrenia version III: A master mechanism. *Molecular Psychiatry*, *28*(5), 1843–1856. https://doi.org/10.1038/s41380-023-02043-w

Howrigan, D. P., Rose, S. A., Samocha, K. E., et al. (2020). Exome sequencing in schizophrenia-affected parent–offspring trios reveals risk conferred by protein-coding de novo mutations. *Nature Neuroscience*, *23*(2), 185–193. https://doi.org/10.1038/s41593-019-0564-3

Huang, H.-S., & Akbarian, S. (2007). GAD1 mRNA Expression and DNA Methylation in Prefrontal Cortex of Subjects with Schizophrenia. *PLOS ONE*, *2*(8), e809. https://doi.org/10.1371/journal.pone.0000809

Huckins, L. M., Dobbyn, A., Ruderfer, D. M., et al. (2019). Gene expression imputation across multiple brain regions provides insights into schizophrenia risk. *Nature Genetics*, *51*(4), 659–674. https://doi.org/10.1038/s41588-019-0364-4

Hurst, C., Pugh, D. A., Abreha, M. H., et al. (2023). Integrated Proteomics to Understand the Role of Neuritin (NRN1) as a Mediator of Cognitive Resilience to Alzheimer's Disease. *Molecular & Cellular Proteomics*, *22*(5). https://doi.org/10.1016/j.mcpro.2023.100542

Ioakeimidis, V., Haenschel, C., Fett, A.-K., et al. (2022). Functional neurodevelopment of working memory in early-onset schizophrenia: A longitudinal FMRI study. *Schizophrenia Research: Cognition*, *30*, 100268. https://doi.org/10.1016/j.scog.2022.100268

Iwamoto, K., Bundo, M., Yamada, K., et al. (2005). DNA Methylation Status of SOX10 Correlates with Its Downregulation and Oligodendrocyte Dysfunction in Schizophrenia. *The Journal of Neuroscience*, *25*(22), 5376–5381. https://doi.org/10.1523/JNEUROSCI.0766-05.2005

Jaenisch, R., & Bird, A. (2003). Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. *Nature Genetics*, *33*(3), 245–254. https://doi.org/10.1038/ng1089

Jagannath, V., Gerstenberg, M., Correll, C. U., et al. (2018). A systematic meta-analysis of the association of Neuregulin 1 (NRG1), D-amino acid oxidase (DAO), and DAO activator (DAOA)/G72 polymorphisms with schizophrenia. *Journal of Neural Transmission (Vienna, Austria: 1996)*, *125*(1), 89–102. https://doi.org/10.1007/s00702-017-1782-z

Janecka, M., Mill, J., Basson, M. A., et al. (2017). Advanced paternal age effects in neurodevelopmental disorders—Review of potential underlying mechanisms. *Translational Psychiatry*, *7*(1), e1019–e1019. https://doi.org/10.1038/tp.2016.294

Jansen, A. G., Mous, S. E., White, T., et al. (2015). What Twin Studies Tell Us About the Heritability of Brain Development, Morphology, and Function: A Review. *Neuropsychology Review*, *25*(1), 27–46. https://doi.org/10.1007/s11065-015-9278-9

Javaherian, A., & Cline, H. T. (2005). Coordinated motor neuron axon growth and neuromuscular synaptogenesis are promoted by CPG15 in vivo. *Neuron*, *45*(4), 505–512. https://doi.org/10.1016/j.neuron.2004.12.051

Ji, Y., Zhang, X., Wang, Z., et al. (2021). Genes associated with gray matter volume alterations in schizophrenia. *NeuroImage*, *225*, 117526. https://doi.org/10.1016/j.neuroimage.2020.117526

Jia, N., Li, Z., Li, X., et al. (2022). Long-term effects of antipsychotics on mortality in patients with schizophrenia: A systematic review and meta-analysis. *Brazilian Journal of Psychiatry*, *44*(6), 664. https://doi.org/10.47626/1516-4446-2021-2306

Jiang, W., King, T. Z., & Turner, J. A. (2019). Imaging Genetics Towards a Refined Diagnosis of Schizophrenia. *Frontiers in Psychiatry*, *10*, 494. https://doi.org/10.3389/fpsyt.2019.00494

Jirtle, R. L., & Skinner, M. K. (2007). Environmental epigenomics and disease susceptibility. *Nature Reviews. Genetics*, *8*(4), 253–262. https://doi.org/10.1038/nrg2045

Jongsma, H. E., Turner, C., Kirkbride, J. B., et al. (2019). International incidence of psychotic disorders, 2002-17: A systematic review and meta-analysis. *The Lancet. Public Health*, *4*(5), e229–e244. https://doi.org/10.1016/S2468-2667(19)30056-8

Jundong, J., Kuja-Halkola, R., Hultman, C., et al. (2011). Poor school performance in offspring of patients with schizophrenia: What are the mechanisms? *Psychological Medicine*, *42*(1), 111. https://doi.org/10.1017/S0033291711001127

Juola, P., Miettunen, J., Veijola, J., et al. (2013). Predictors of short – and long-term clinical outcome in schizophrenic psychosis – the Northern Finland 1966 Birth Cohort study. *European Psychiatry*, *28*(5), 263–268. https://doi.org/10.1016/j.eurpsy.2011.11.001

Kahn, R. S., & Keefe, R. S. E. (2013). Schizophrenia Is a Cognitive Illness: Time for a Change in Focus. *JAMA Psychiatry*, *70*(10), 1107–1112. https://doi.org/10.1001/jamapsychiatry.2013.155

Kaldun, J. C., & Sprecher, S. G. (2019). Initiated by CREB: Resolving Gene Regulatory Programs in Learning and Memory. *BioEssays*, *41*(8), 1900045. https://doi.org/10.1002/bies.201900045

Kalinowska, S., Trześniowska-Drukała, B., Kłoda, K., et al. (2021). The Association between Lifestyle Choices and Schizophrenia Symptoms. *Journal of Clinical Medicine*, *10*(1), 165. https://doi.org/10.3390/jcm10010165

Kane, M. J., Conway, A. R. A., Miura, T. K., et al. (2007). Working memory, attention control, and the n-back task: A question of construct validity. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *33*(3), 615–622. https://doi.org/10.1037/0278-7393.33.3.615

Kang, H. J., Kawasawa, Y. I., Cheng, F., et al. (2011). Spatio-temporal transcriptome of the human brain. *Nature*, *478*(7370), 483–489. https://doi.org/10.1038/nature10523

Kapur, S., & Remington, G. (2001). Dopamine D(2) receptors and their role in atypical antipsychotic action: Still necessary and may even be sufficient. *Biological Psychiatry*, *50*(11), 873–883. https://doi.org/10.1016/s0006-3223(01)01251-3

Kapur, S., & Seeman, P. (2001). Does fast dissociation from the dopamine d(2) receptor explain the action of atypical antipsychotics?: A new hypothesis. *The American Journal of Psychiatry*, *158*(3), 360–369. https://doi.org/10.1176/appi.ajp.158.3.360

Karamoysoyli, E., Burnand, R. C., Tomlinson, D. R., et al. (2008). Neuritin mediates nerve growth factor-induced axonal regeneration and is deficient in experimental diabetic neuropathy. *Diabetes*, *57*(1), 181–189. https://doi.org/10.2337/db07-0895

Katirtzoglou, A., Hansen, S. B., Sveier, H., et al. (2024). Genomic context determines the effect of DNA methylation on gene expression in the gut epithelium of Atlantic salmon (Salmo salar). *Epigenetics*, *19*(1), 2392049. https://doi.org/10.1080/15592294.2024.2392049

Kay, S. R., Fiszbein, A., & Opler, L. A. (1987). The Positive and Negative Syndrome Scale (PANSS) for Schizophrenia. *Schizophrenia Bulletin*, *13*(2), 261–276. https://doi.org/10.1093/schbul/13.2.261

Kebir, O., Chaumette, B., Rivollier, F., et al. (2017). Methylomic changes during conversion to psychosis. *Molecular Psychiatry*, *22*(4), 512–518. https://doi.org/10.1038/mp.2016.53

Keefe, R. S. E., Fox, K. H., Harvey, P. D., et al. (2011). Characteristics of the MATRICS Consensus Cognitive Battery in a 29-site antipsychotic schizophrenia clinical trial. *Schizophrenia Research*, *125*(2–3), 161–168. https://doi.org/10.1016/j.schres.2010.09.015

Keifer Jr, O. P., Hurt, R. C., Gutman, D. A., et al. (2015). Voxel-based morphometry predicts shifts in dendritic spine density and morphology with auditory fear conditioning. *Nature Communications*, *6*(1), 7582. https://doi.org/10.1038/ncomms8582

Kendell, R., & Jablensky, A. (2003). Distinguishing Between the Validity and Utility of Psychiatric Diagnoses. *American Journal of Psychiatry*, *160*(1), 4–12. https://doi.org/10.1176/appi.ajp.160.1.4

Kendler, K. S., Ohlsson, H., Mezuk, B., et al. (2016). Observed Cognitive Performance and Deviation From Familial Cognitive Aptitude at Age 16 Years and Ages 18 to 20 Years and Risk for Schizophrenia and Bipolar Illness in a Swedish National Sample. *JAMA Psychiatry*, *73*(5), 465–471. https://doi.org/10.1001/jamapsychiatry.2016.0053

Kern, R. S., Nuechterlein, K. H., Green, M. F., et al. (2008). The MATRICS Consensus Cognitive Battery, part 2: Co-norming and standardization. *The American Journal of Psychiatry*, *165*(2), 214–220. https://doi.org/10.1176/appi.ajp.2007.07010043

Kesby, J. P., Eyles, D. W., McGrath, J. J., et al. (2018). Dopamine, psychosis and schizophrenia: The widening gap between basic and clinical neuroscience. *Translational Psychiatry*, *8*(1), 1–12. https://doi.org/10.1038/s41398-017-0071-9

Khachadourian, V., Zaks, N., Lin, E., et al. (2021). Advanced paternal age and risk of schizophrenia in offspring–review of epidemiological findings and potential mechanisms. *Schizophrenia Research*, *233*, 72–79. https://doi.org/10.1016/j.schres.2021.06.016

Khandaker, G. M., Barnett, J. H., White, I. R., et al. (2011). A quantitative meta-analysis of population-based studies of premorbid intelligence and schizophrenia. *Schizophrenia Research*, *132*(2–3), 220–227. https://doi.org/10.1016/j.schres.2011.06.017

Khodosevich, K., & Sellgren, C. M. (2023). Neurodevelopmental disorders—High-resolution rethinking of disease modeling. *Molecular Psychiatry*, *28*(1), 34–43. https://doi.org/10.1038/s41380-022-01876-1

Kim, S.-H., & Park, S. (2023). Effectiveness of family interventions for patients with schizophrenia: A systematic review and meta-analysis. *International Journal of Mental Health Nursing*, *32*(6), 1598–1615. https://doi.org/10.1111/inm.13198

Kinon, B. J., Chen, L., Ascher-Svanum, H., et al. (2010). Early response to antipsychotic drug therapy as a clinical marker of subsequent response in the treatment of schizophrenia.

Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, *35*(2), 581–590. https://doi.org/10.1038/npp.2009.164

Kirschner, M., Shafiei, G., Markello, R. D., et al. (2020). Latent Clinical-Anatomical Dimensions of Schizophrenia. *Schizophrenia Bulletin*, *46*(6), 1426–1438. https://doi.org/10.1093/schbul/sbaa097

Kordi-Tamandani, D. M., Sahranavard, R., & Torkamanzehi, A. (2013). Analysis of association between dopamine receptor genes' methylation and their expression profile with the risk of schizophrenia. *Psychiatric Genetics*, *23*(5), 183. https://doi.org/10.1097/YPG.0b013e328363d6e1

Kozak, M. J., & Cuthbert, B. N. (2016). The NIMH Research Domain Criteria Initiative: Background, Issues, and Pragmatics. *Psychophysiology*, *53*(3), 286–297. https://doi.org/10.1111/psyp.12518

Kruse, A. O., & Bustillo, J. R. (2022). Glutamatergic dysfunction in Schizophrenia. *Translational Psychiatry*, *12*(1), 1–13. https://doi.org/10.1038/s41398-022-02253-w

Krystal, J. H., Karper, L. P., Seibyl, J. P., et al. (1994). Subanesthetic Effects of the Noncompetitive NMDA Antagonist, Ketamine, in Humans: Psychotomimetic, Perceptual, Cognitive, and Neuroendocrine Responses. *Archives of General Psychiatry*, *51*(3), 199–214. https://doi.org/10.1001/archpsyc.1994.03950030035004

Kundakovic, M., Chen, Y., Costa, E., et al. (2007). DNA Methyltransferase Inhibitors Coordinately Induce Expression of the Human Reelin and Glutamic Acid Decarboxylase 67 Genes. *Molecular Pharmacology*, *71*(3), 644–653. https://doi.org/10.1124/mol.106.030635

Kung, L., Conley, R., Chute, D. J., et al. (1998). Synaptic changes in the striatum of schizophrenic cases: A controlled postmortem ultrastructural study. *Synapse*, *28*(2), 125–139. https://doi.org/10.1002/(SICI)1098-2396(199802)28:2<125::AID-SYN3>3.0.CO;2-D

Kuo, S. S., & Pogue-Geile, M. F. (2019). Variation in Fourteen Brain Structure Volumes in Schizophrenia: A Comprehensive Meta-Analysis of 246 Studies. *Neuroscience and Biobehavioral Reviews*, *98*, 85–94. https://doi.org/10.1016/j.neubiorev.2018.12.030

Kyriakopoulos, M., Dima, D., Roiser, J. P., et al. (2012). Abnormal functional activation and connectivity in the working memory network in early-onset schizophrenia. *Journal of the American Academy of Child and Adolescent Psychiatry*, *51*(9), 911-920.e2. https://doi.org/10.1016/j.jaac.2012.06.020

Larsen, T. K., Friis, S., Haahr, U., et al. (2004). Premorbid adjustment in first-episode non-affective psychosis: Distinct patterns of pre-onset course. *The British Journal of Psychiatry*, *185*(2), 108–115. https://doi.org/10.1192/bjp.185.2.108

Laruelle, M., & Abi-Dargham, A. (1999). Dopamine as the wind of the psychotic fire: New evidence from brain imaging studies. *Journal of Psychopharmacology (Oxford, England)*, *13*(4), 358–371. https://doi.org/10.1177/026988119901300405

Le, L., R, K., B, M., et al. (2020). Risk of schizophrenia in relatives of individuals affected by schizophrenia: A meta-analysis. *Psychiatry Research*, *286*, 112852. https://doi.org/10.1016/j.psychres.2020.112852

Lean, M., Fornells-Ambrojo, M., Milton, A., et al. (2019). Self-management interventions for people with severe mental illness: Systematic review and meta-analysis. *The British Journal of Psychiatry*, *214*(5), 260. https://doi.org/10.1192/bjp.2019.54

Leaune, E., Dealberto, M.-J., Luck, D., et al. (2019). Ethnic minority position and migrant status as risk factors for psychotic symptoms in the general population: A meta-analysis. *Psychological Medicine*, *49*(4), 545–558. https://doi.org/10.1017/S0033291718002271

Lee, B. J., Marchionni, L., Andrews, C. E., et al. (2017). Analysis of differential gene expression mediated by clozapine in human postmortem brains. *Schizophrenia Research*, *185*, 58–66. https://doi.org/10.1016/j.schres.2016.12.017

Lee, K.-H., Ryu, C. J., Hong, H. J., et al. (2005). cDNA Microarray Analysis of Nerve Growth Factor-Regulated Gene Expression Profile in Rat PC12 Cells. *Neurochemical Research*, *30*(4), 533–540. https://doi.org/10.1007/s11064-005-2688-y

Lee, W.-C. A., & Nedivi, E. (2002). Extended Plasticity of Visual Cortex in Dark-Reared Animals May Result from Prolonged Expression of cpg15-Like Genes. *The Journal of Neuroscience*, *22*(5), 1807–1815. https://doi.org/10.1523/JNEUROSCI.22-05-01807.2002

Lefebvre, J. L., Sanes, J. R., & Kay, J. N. (2015). Development of dendritic form and function. *Annual Review of Cell and Developmental Biology*, *31*, 741–777. https://doi.org/10.1146/annurev-cellbio-100913-013020

Leger, M., & Neill, J. C. (2016). A systematic review comparing sex differences in cognitive function in schizophrenia and in rodent models for schizophrenia, implications for improved therapeutic strategies. *Neuroscience and Biobehavioral Reviews*, *68*, 979–1000. https://doi.org/10.1016/j.neubiorev.2016.06.029

Legge, S. E., Cardno, A. G., Allardyce, J., et al. (2021). Associations Between Schizophrenia Polygenic Liability, Symptom Dimensions, and Cognitive Ability in Schizophrenia. *JAMA Psychiatry*, *78*(10), 1143–1151. https://doi.org/10.1001/jamapsychiatry.2021.1961

Legge, S. E., Santoro, M. L., Periyasamy, S., et al. (2021). Genetic architecture of schizophrenia: A review of major advancements. *Psychological Medicine*, *51*(13), 2168–2177. https://doi.org/10.1017/S0033291720005334

Lencz, T., Knowles, E., Davies, G., et al. (2013). Molecular Genetic Evidence for Genetic Overlap between General Cognitive Ability and Risk for Schizophrenia: A Report from the Cognitive Genomics Consortium (COGENT). *Molecular Psychiatry*, *19*(2), 168. https://doi.org/10.1038/mp.2013.166

Lenroot, R. K., & Giedd, J. N. (2006). Brain development in children and adolescents: Insights from anatomical magnetic resonance imaging. *Neuroscience & Biobehavioral Reviews*, *30*(6), 718–729. https://doi.org/10.1016/j.neubiorev.2006.06.001

Leucht, S., Cipriani, A., Spineli, L., et al. (2013). Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: A multiple-treatments meta-analysis. *Lancet (London, England)*, *382*(9896), 951–962. https://doi.org/10.1016/S0140-6736(13)60733-3

Levenson, J. M., Roth, T. L., Lubin, F. D., et al. (2006). Evidence That DNA (Cytosine-5) Methyltransferase Regulates Synaptic Plasticity in the Hippocampus *. *Journal of Biological Chemistry*, *281*(23), 15763–15773. https://doi.org/10.1074/jbc.M511767200

Li, M., Chang, H., & Xiao, X. (2016). BDNF Val66Met polymorphism and bipolar disorder in European populations: A risk association in case-control, family-based and GWAS studies. *Neuroscience & Biobehavioral Reviews*, *68*, 218–233. https://doi.org/10.1016/j.neubiorev.2016.05.031

Lichtenstein, P., Yip, B. H., Björk, C., et al. (2009). Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: A population-based study. *The Lancet*, *373*(9659), 234–239. https://doi.org/10.1016/S0140-6736(09)60072-6

Lincoln, S. H., Johnson, T., Winters, A., et al. (2021). Social exclusion and rejection across the psychosis spectrum: A systematic review of empirical research. *Schizophrenia Research*, *228*, 43–50. https://doi.org/10.1016/j.schres.2020.11.056

Lindholm, E., Ekholm, B., Shaw, S., et al. (2001). A Schizophrenia-Susceptibility Locus at 6q25, in One of the World's Largest Reported Pedigrees. *The American Journal of Human Genetics*, *69*(1), 96–105. https://doi.org/10.1086/321288

Linhares, N. D., Svartman, M., Rodrigues, T. C., et al. (2015). Subtelomeric 6p25 deletion/duplication: Report of a patient with new clinical findings and genotype–phenotype correlations. *European Journal of Medical Genetics*, *58*(5), 310–318. https://doi.org/10.1016/j.ejmg.2015.02.011

Lister, R., Mukamel, E. A., Nery, J. R., et al. (2013). Global Epigenomic Reconfiguration During Mammalian Brain Development. *Science*, *341*(6146), 1237905. https://doi.org/10.1126/science.1237905

Liu, Q., Zhang, H., Xu, J., et al. (2018). Neuritin provides neuroprotection against experimental traumatic brain injury in rats. *International Journal of Neuroscience*, *128*(9), 811–820. https://doi.org/10.1080/00207454.2018.1424155

Liu, Y., Lichtenstein, P., Kotov, R., et al. (2024). Exploring the genetic etiology across the continuum of the general psychopathology factor: A Swedish population-based family and twin study. *Molecular Psychiatry*, *29*(10), 2921–2928. https://doi.org/10.1038/s41380-024-02552-2

Llorca, A., & Marín, O. (2021). Orchestrated freedom: New insights into cortical neurogenesis. *Current Opinion in Neurobiology*, *66*, 48–56. https://doi.org/10.1016/j.conb.2020.09.004

Loeb, F. F., Zhou, X., Craddock, K. E. S., et al. (2018). Reduced Functional Brain Activation and Connectivity During a Working Memory Task in Childhood-Onset Schizophrenia. *Journal of the American Academy of Child & Adolescent Psychiatry*, *57*(3), 166–174. https://doi.org/10.1016/j.jaac.2017.12.009

Lu, J.-M., Liu, D.-D., Li, Z.-Y., et al. (2017). Neuritin Enhances Synaptic Transmission in Medial Prefrontal Cortex in Mice by Increasing CaV3.3 Surface Expression. *Cerebral Cortex*, *27*(7), 3842–3855. https://doi.org/10.1093/cercor/bhx082

Lu, L., Dong, M., Zhang, L., et al. (2019). Prevalence of suicide attempts in individuals with schizophrenia: A meta-analysis of observational studies. *Epidemiology and Psychiatric Sciences*, *29*, e39. https://doi.org/10.1017/S2045796019000313

Luciano, A., Bond, G. R., & Drake, R. E. (2014). Does employment alter the course and outcome of schizophrenia and other severe mental illnesses? A systematic review of longitudinal research. *Schizophrenia Research*, *159*(2), 312–321. https://doi.org/10.1016/j.schres.2014.09.010

Lund, C., Brooke-Sumner, C., Baingana, F., et al. (2018). Social determinants of mental disorders and the Sustainable Development Goals: A systematic review of reviews. *The Lancet Psychiatry*, *5*(4), 357–369. https://doi.org/10.1016/S2215-0366(18)30060-9

Luo, S. X., & Huang, E. J. (2016). Dopaminergic Neurons and Brain Reward Pathways: From Neurogenesis to Circuit Assembly. *The American Journal of Pathology*, *186*(3), 478–488. https://doi.org/10.1016/j.ajpath.2015.09.023

Ma, H., Khaled, H. G., Wang, X., et al. (2023). Excitation–transcription coupling, neuronal gene expression and synaptic plasticity. *Nature Reviews Neuroscience*, *24*(11), 672–692. https://doi.org/10.1038/s41583-023-00742-5

Mabry, S. J., McCollum, L. A., Farmer, C. B., et al. (2020). Evidence for altered excitatory and inhibitory tone in the postmortem substantia nigra in schizophrenia. *The World Journal of Biological Psychiatry : The Official Journal of the World Federation of Societies of Biological Psychiatry*, *21*(5), 339–356. https://doi.org/10.1080/15622975.2019.1615638

MacCabe, J. H., Lambe, M. P., Cnattingius, S., et al. (2008). Scholastic achievement at age 16 and risk of schizophrenia and other psychoses: A national cohort study. *Psychological Medicine*, *38*(8), 1133–1140. https://doi.org/10.1017/S0033291707002048

Magee, J. C., & Grienberger, C. (2020). Synaptic Plasticity Forms and Functions. *Annual Review of Neuroscience*, *43*(Volume 43, 2020), 95–117. https://doi.org/10.1146/annurev-neuro-090919-022842

Malaspina, D., Goetz, R. R., Yale, S., et al. (2000). Relation of Familial Schizophrenia to Negative Symptoms But Not to the Deficit Syndrome. *American Journal of Psychiatry*, *157*(6), 994–1003. https://doi.org/10.1176/appi.ajp.157.6.994

Mané, A., Bergé, D., Penzol, M. J., et al. (2017). Cannabis use, COMT, BDNF and age at first-episode psychosis. *Psychiatry Research*, *250*, 38–43. https://doi.org/10.1016/j.psychres.2017.01.045

Mannarini, S., Taccini, F., Sato, I., et al. (2022). Understanding stigma toward schizophrenia. *Psychiatry Research*, *318*, 114970. https://doi.org/10.1016/j.psychres.2022.114970

Marron, T. U., Guerini, V., Rusmini, P., et al. (2005). Androgen-induced neurite outgrowth is mediated by neuritin in motor neurones. *Journal of Neurochemistry*, *92*(1), 10–20. https://doi.org/10.1111/j.1471-4159.2004.02836.x

Marshall, C., Denny, E., Cadenhead, K., et al. (2014). The content of attenuated psychotic symptoms in those at clinical high risk for psychosis. *Psychiatry Research*, *219*(3), 506–512. https://doi.org/10.1016/j.psychres.2014.06.023

Marshall, C. R., Howrigan, D. P., Merico, D., et al. (2017). Contribution of copy number variants to schizophrenia from a genome-wide study of 41,321 subjects. *Nature Genetics*, *49*(1), 27–35. https://doi.org/10.1038/ng.3725

Martel, J. C., & Gatti McArthur, S. (2020). Dopamine Receptor Subtypes, Physiology and Pharmacology: New Ligands and Concepts in Schizophrenia. *Frontiers in Pharmacology*, *11*. https://doi.org/10.3389/fphar.2020.01003

Martinet, D., Filges, I., Besuchet Schmutz, N., et al. (2008). Subtelomeric 6p deletion: Clinical and array-CGH characterization in two patients. *American Journal of Medical Genetics Part A*, *146A*(16), 2094–2102. https://doi.org/10.1002/ajmg.a.32414

Marzola, P., Melzer, T., Pavesi, E., et al. (2023). Exploring the Role of Neuroplasticity in Development, Aging, and Neurodegeneration. *Brain Sciences*, *13*(12), 1610. https://doi.org/10.3390/brainsci13121610

Mas-Bermejo, P., Papiol, S., Torrecilla, P., et al. (2025). Sex-specific association between schizophrenia polygenic risk and subclinical schizophrenia-related traits. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *136*, 111161. https://doi.org/10.1016/j.pnpbp.2024.111161

Matsuoka, K., Morimoto, T., Matsuda, Y., et al. (2019). Computer-assisted cognitive remediation therapy for patients with schizophrenia induces microstructural changes in cerebellar regions involved in cognitive functions. *Psychiatry Research: Neuroimaging*, *292*, 41–46. https://doi.org/10.1016/j.pscychresns.2019.09.001

Mazin, P., Xiong, J., Liu, X., et al. (2013). Widespread splicing changes in human brain development and aging. *Molecular Systems Biology*, *9*, 633. https://doi.org/10.1038/msb.2012.67

McCullumsmith, R. E., Hammond, J. H., Shan, D., et al. (2014). Postmortem Brain: An Underutilized Substrate for Studying Severe Mental Illness. *Neuropsychopharmacology*, *39*(1), 65–87. https://doi.org/10.1038/npp.2013.239

McCutcheon, R. A., Reis Marques, T., & Howes, O. D. (2020). Schizophrenia—An Overview. *JAMA Psychiatry*, *77*(2), 201–210. https://doi.org/10.1001/jamapsychiatry.2019.3360

McGrath, J., El-Saadi, O., Grim, V., et al. (2002). Minor Physical Anomalies and Quantitative Measures of the Head and Face in Patients With Psychosis. *Archives of General Psychiatry*, *59*(5), 458–464. https://doi.org/10.1001/archpsyc.59.5.458

McKetin, R., Leung, J., Stockings, E., et al. (2019). Mental health outcomes associated with the use of amphetamines: A systematic review and meta-analysis. *eClinicalMedicine*, *16*, 81–97. https://doi.org/10.1016/j.eclinm.2019.09.014

McKinney, B., Ding, Y., Lewis, D. A., et al. (2017). DNA methylation as a putative mechanism for reduced dendritic spine density in the superior temporal gyrus of subjects with schizophrenia. *Translational Psychiatry*, *7*(2), e1032–e1032. https://doi.org/10.1038/tp.2016.297

Meier, M. H., Caspi, A., Reichenberg, A., et al. (2014). Neuropsychological Decline in Schizophrenia from the Premorbid to Post-Onset Period: Evidence from a Population-Representative Longitudinal Study. *The American Journal of Psychiatry*, *171*(1), 91–101. https://doi.org/10.1176/appi.ajp.2013.12111438

Meijer, M., Keo, A., van Leeuwen, J. M. C., et al. (2021). Molecular characterization of the stress network in individuals at risk for schizophrenia. *Neurobiology of Stress*, *14*, 100307. https://doi.org/10.1016/j.ynstr.2021.100307

Merikangas, A. K., Shelly, M., Knighton, A., et al. (2022). What genes are differentially expressed in individuals with schizophrenia? A systematic review. *Molecular Psychiatry*, *27*(3), 1373–1383. https://doi.org/10.1038/s41380-021-01420-7

Miller, C. A., & Sweatt, J. D. (2007). Covalent Modification of DNA Regulates Memory Formation. *Neuron*, *53*(6), 857–869. https://doi.org/10.1016/j.neuron.2007.02.022

Miller, D. J., Duka, T., Stimpson, C. D., et al. (2012). Prolonged myelination in human neocortical evolution. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(41), 16480–16485. https://doi.org/10.1073/pnas.1117943109

Miller, J. A., Scult, M. A., Conley, E. D., et al. (2018). Effects of Schizophrenia Polygenic Risk Scores on Brain Activity and Performance During Working Memory Subprocesses in Healthy Young Adults. *Schizophrenia Bulletin*, *44*(4), 844–853. https://doi.org/10.1093/schbul/sbx140

Mira, H., & Morante, J. (2020). Neurogenesis From Embryo to Adult – Lessons From Flies and Mice. *Frontiers in Cell and Developmental Biology*, *8*. https://doi.org/10.3389/fcell.2020.00533

Miranda, G. G., Rodrigue, K. M., & Kennedy, K. M. (2019). Fronto-parietal cortical thickness mediates the effect of COMT Val158Met polymorphism on age-associated executive function. *Neurobiology of Aging*, *73*, 104–114. https://doi.org/10.1016/j.neurobiolaging.2018.08.027

Mohamed, Z. I., Tee, S. F., & Tang, P. Y. (2018). Association of functional polymorphisms in 3'-untranslated regions of COMT, DISC1, and DTNBP1 with schizophrenia: A meta-analysis. *Psychiatric Genetics*, *28*(6), 110–119. https://doi.org/10.1097/YPG.0000000000000210

Moises, H. W., Yang, L., Kristbjarnarson, H., et al. (1995). An international two–stage genome–wide search for schizophrenia susceptibility genes. *Nature Genetics*, *11*(3), 321–324. https://doi.org/10.1038/ng1195-321

Molofsky, A. V., Krenick, R., Ullian, E., et al. (2012). Astrocytes and disease: A neurodevelopmental perspective. *Genes & Development*, *26*(9), 891. https://doi.org/10.1101/gad.188326.112

Montano, C., Taub, M. A., Jaffe, A., et al. (2016). Association of DNA Methylation Differences With Schizophrenia in an Epigenome-Wide Association Study. *JAMA Psychiatry*, *73*(5), 506–514. https://doi.org/10.1001/jamapsychiatry.2016.0144

Moore, L. D., Le, T., & Fan, G. (2013). DNA Methylation and Its Basic Function. *Neuropsychopharmacology*, *38*(1), 23–38. https://doi.org/10.1038/npp.2012.112

Mordelt, A., & de Witte, L. D. (2023). Microglia-mediated synaptic pruning as a key deficit in neurodevelopmental disorders: Hype or hope? *Current Opinion in Neurobiology*, *79*, 102674. https://doi.org/10.1016/j.conb.2022.102674

Morimoto, T., Matsuda, Y., Matsuoka, K., et al. (2018). Computer-assisted cognitive remediation therapy increases hippocampal volume in patients with schizophrenia: A randomized controlled trial. *BMC Psychiatry*, *18*(1), 83. https://doi.org/10.1186/s12888-018-1667-1

Mothersill, D., & Donohoe, G. (2019). Neural Effects of Cognitive Training in Schizophrenia: A Systematic Review and Activation Likelihood Estimation Meta-analysis. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *4*(8), 688–696. https://doi.org/10.1016/j.bpsc.2019.03.005

Moura, B. M., van Rooijen, G., Schirmbeck, F., et al. (2021). A Network of Psychopathological, Cognitive, and Motor Symptoms in Schizophrenia Spectrum Disorders. *Schizophrenia Bulletin*, *47*(4), 915–926. https://doi.org/10.1093/schbul/sbab002

Murao, N., Noguchi, H., & Nakashima, K. (2016). Epigenetic regulation of neural stem cell property from embryo to adult. *Neuroepigenetics*, *5*, 1–10. https://doi.org/10.1016/j.nepig.2016.01.001

Naeve, G. S., Ramakrishnan, M., Kramer, R., et al. (1997). Neuritin: A gene induced by neural activity and neurotrophins that promotes neuritogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(6), 2648–2653.

Nagasaka, K., Nemoto, K., Takashima, I., et al. (2021). Structural Plastic Changes of Cortical Gray Matter Revealed by Voxel-Based Morphometry and Histological Analyses in a Monkey Model of Central Post-Stroke Pain. *Cerebral Cortex (New York, N.Y.: 1991)*, *31*(10), 4439–4449. https://doi.org/10.1093/cercor/bhab098

Nani, J. V., Muotri, A. R., & Hayashi, M. A. F. (2024). Peering into the mind: Unraveling schizophrenia's secrets using models. *Molecular Psychiatry*, 1–20. https://doi.org/10.1038/s41380-024-02728-w

Nascimento, J. M., & Martins-de-Souza, D. (2015). The proteome of schizophrenia. *Npj Schizophrenia*, *1*(1), Article 1. https://doi.org/10.1038/npjschz.2014.3

Nedivi, E., Fieldust, S., Theill, L. E., et al. (1996). A set of genes expressed in response to light in the adult cerebral cortex and regulated during development. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(5), 2048–2053.

Nedivi, E., Javaherian, A., Cantallops, I., et al. (2001). Developmental Regulation of CPG15 Expression in Xenopus. *The Journal of Comparative Neurology*, *435*(4), 464–473.

Nedivi, E., Wu, G.-Y., & Cline, H. T. (1998). Promotion of Dendritic Growth by CPG15, an Activity-Induced Signaling Molecule. *Science (New York, N.Y.)*, *281*(5384), 1863–1866.

Negi, S. K., & Guda, C. (2017). Global gene expression profiling of healthy human brain and its application in studying neurological disorders. *Scientific Reports*, *7*(1), 897. https://doi.org/10.1038/s41598-017-00952-9

Newton, S. S., Collier, E. F., Hunsberger, J., et al. (2003). Gene Profile of Electroconvulsive Seizures: Induction of Neurotrophic and Angiogenic Factors. *The Journal of Neuroscience*, *23*(34), 10841–10851. https://doi.org/10.1523/JNEUROSCI.23-34-10841.2003

Ni, G., Gratten, J., Wray, N. R., et al. (2018). Age at first birth in women is genetically associated with increased risk of schizophrenia. *Scientific Reports*, *8*(1), 10168. https://doi.org/10.1038/s41598-018-28160-z

Nieto, R. R., Carrasco, A., Corral, S., et al. (2021). BDNF as a Biomarker of Cognition in Schizophrenia/Psychosis: An Updated Review. *Frontiers in Psychiatry*, *12*. https://doi.org/10.3389/fpsyt.2021.662407

Notaras, M., Lodhi, A., Dündar, F., et al. (2022). Schizophrenia is defined by cell-specific neuropathology and multiple neurodevelopmental mechanisms in patient-derived cerebral organoids. *Molecular Psychiatry*, *27*(3), 1416–1434. https://doi.org/10.1038/s41380-021-01316-6

Nuechterlein, K. H., Green, M. F., Kern, R. S., et al. (2008). The MATRICS Consensus Cognitive Battery, part 1: Test selection, reliability, and validity. *The American Journal of Psychiatry*, *165*(2), 203–213. https://doi.org/10.1176/appi.ajp.2007.07010042

O'Donoghue, B., Roche, E., & Lane, A. (2016). Neighbourhood level social deprivation and the risk of psychotic disorders: A systematic review. *Social Psychiatry and Psychiatric Epidemiology*, *51*(7), 941–950. https://doi.org/10.1007/s00127-016-1233-4

Ohi, K., Ochi, R., Noda, Y., et al. (2021). Polygenic risk scores for major psychiatric and neurodevelopmental disorders contribute to sleep disturbance in childhood: Adolescent Brain Cognitive Development (ABCD) Study. *Translational Psychiatry*, *11*, 187. https://doi.org/10.1038/s41398-021-01308-8

Ojeda, N., Peña, J., Schretlen, D. J., et al. (2012). Hierarchical structure of the cognitive processes in schizophrenia: The fundamental role of processing speed. *Schizophrenia Research*, *135*(1–3), 72–78. https://doi.org/10.1016/j.schres.2011.12.004

Oord, E. J. C. G. van den, Clark, S. L., Xie, L. Y., et al. (2015). A Whole Methylome CpG-SNP Association Study of Psychosis in Blood and Brain Tissue. *Schizophrenia Bulletin*, *42*(4), 1018. https://doi.org/10.1093/schbul/sbv182

Osimo, E. F., Beck, K., Reis Marques, T., et al. (2019). Synaptic loss in schizophrenia: A meta-analysis and systematic review of synaptic protein and mRNA measures. *Molecular Psychiatry*, *24*(4), 549–561. https://doi.org/10.1038/s41380-018-0041-5

Owen, M. J. (2015). Psychotic Disorders and the Neurodevelopmental Continuum. In K. Nikolich & S. E. Hyman (Eds.), *Translational Neuroscience: Toward New Therapies*. MIT Press. http://www.ncbi.nlm.nih.gov/books/NBK569704/

Owen, M. J., Legge, S. E., Rees, E., et al. (2023). Genomic findings in schizophrenia and their implications. *Molecular Psychiatry*, *28*(9), 3638–3647. https://doi.org/10.1038/s41380-023-02293-8

Pardiñas, A. F., Holmans, P., Pocklington, A. J., et al. (2018). Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection. *Nature Genetics*, *50*(3), Article 3. https://doi.org/10.1038/s41588-018-0059-2

Park, H. G., Yu, H. S., Park, S., et al. (2014). Repeated treatment with electroconvulsive seizures induces HDAC2 expression and down-regulation of NMDA receptor-related genes through histone deacetylation in the rat frontal cortex. *International Journal of Neuropsychopharmacology*, *17*(9), 1487–1500. https://doi.org/10.1017/S1461145714000248

Park, S., Holzman, P. S., & Goldman-Rakic, P. S. (1995). Spatial working memory deficits in the relatives of schizophrenic patients. *Archives of General Psychiatry*, *52*(10), 821–828. https://doi.org/10.1001/archpsyc.1995.03950220031007

Pauly, K., Seiferth, N. Y., Kellermann, T., et al. (2008). Cerebral Dysfunctions of Emotion—Cognition Interactions in Adolescent-Onset Schizophrenia. *Journal of the American Academy of Child & Adolescent Psychiatry*, *47*(11), 1299–1310. https://doi.org/10.1097/CHI.0b013e318184ff16

Payá, B., Rodríguez-Sánchez, J. M., Otero, S., et al. (2013). Premorbid impairments in early-onset psychosis: Differences between patients with schizophrenia and bipolar disorder. *Schizophrenia Research*, *146*(1), 103–110. https://doi.org/10.1016/j.schres.2013.01.029

Penadés, R., Almodóvar-Payá, C., García-Rizo, C., et al. (2024). Changes in *BDNF* methylation patterns after cognitive remediation therapy in schizophrenia: A randomized and controlled trial. *Journal of Psychiatric Research*, *173*, 166–174. https://doi.org/10.1016/j.jpsychires.2024.03.014

Penadés, R., González-Rodríguez, A., Catalán, R., et al. (2017). Neuroimaging studies of cognitive remediation in schizophrenia: A systematic and critical review. *World Journal of Psychiatry*, *7*(1), 34–43. https://doi.org/10.5498/wjp.v7.i1.34

Penadés, R., Pujol, N., Catalán, R., et al. (2013). Brain Effects of Cognitive Remediation Therapy in Schizophrenia: A Structural and Functional Neuroimaging Study. *Biological Psychiatry*, *73*(10), 1015–1023. https://doi.org/10.1016/j.biopsych.2013.01.017

Penadés, R., Segura, B., Inguanzo, A., et al. (2020). Cognitive remediation and brain connectivity: A resting-state fMRI study in patients with schizophrenia. *Psychiatry Research: Neuroimaging*, *303*, 111140. https://doi.org/10.1016/j.pscychresns.2020.111140

Penisson, M., Ladewig, J., Belvindrah, R., et al. (2019). Genes and Mechanisms Involved in the Generation and Amplification of Basal Radial Glial Cells. *Frontiers in Cellular Neuroscience*, *13*. https://doi.org/10.3389/fncel.2019.00381

Penttilä, M., Jääskeläinen, E., Hirvonen, N., et al. (2014). Duration of untreated psychosis as predictor of long-term outcome in schizophrenia: Systematic review and meta-analysis. *The British Journal of Psychiatry*, *205*(2), 88–94. https://doi.org/10.1192/bjp.bp.113.127753

Perera, B. P. U., Faulk, C., Svoboda, L. K., et al. (2020). The role of environmental exposures and the epigenome in health and disease. *Environmental and Molecular Mutagenesis*, *61*(1), 176–192. https://doi.org/10.1002/em.22311

Perkins, D. O., Olde Loohuis, L., Barbee, J., et al. (2020). Polygenic Risk Score Contribution to Psychosis Prediction in a Target Population of Persons at Clinical High Risk. *The American Journal of Psychiatry*, *177*(2), 155–163. https://doi.org/10.1176/appi.ajp.2019.18060721

Petanjek, Z., Judaš, M., Šimic, G., et al. (2011). Extraordinary neoteny of synaptic spines in the human prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(32), 13281–13286. https://doi.org/10.1073/pnas.1105108108

Petrescu, C., Petrescu, D. M., Marian, G., et al. (2023). Neurological Soft Signs in Schizophrenia, a Picture of the Knowledge in the Last Decade: A Scoping Review. *Healthcare*, *11*(10), Article 10. https://doi.org/10.3390/healthcare11101471

Picardi, A., Viroli, C., Tarsitani, L., et al. (2012). Heterogeneity and symptom structure of schizophrenia. *Psychiatry Research*, *198*(3), 386–394. https://doi.org/10.1016/j.psychres.2011.12.051

Pidsley, R., Viana, J., Hannon, E., et al. (2014). Methylomic profiling of human brain tissue supports a neurodevelopmental origin for schizophrenia. *Genome Biology*, *15*(10), 483. https://doi.org/10.1186/s13059-014-0483-2

Pillinger, T., McCutcheon, R. A., Vano, L., et al. (2020). Comparative effects of 18 antipsychotics on metabolic function in patients with schizophrenia, predictors of metabolic dysregulation, and association with psychopathology: A systematic review and network meta-analysis. *The Lancet. Psychiatry*, *7*(1), 64–77. https://doi.org/10.1016/S2215-0366(19)30416-X

Plana-Ripoll, O., Pedersen, C. B., Holtz, Y., et al. (2019). Exploring Comorbidity Within Mental Disorders Among a Danish National Population. *JAMA Psychiatry*, *76*(3), 259–270. https://doi.org/10.1001/jamapsychiatry.2018.3658

Potkin, S. G., Kane, J. M., Correll, C. U., et al. (2020). The neurobiology of treatment-resistant schizophrenia: Paths to antipsychotic resistance and a roadmap for future research. *Npj Schizophrenia*, *6*(1), 1–10. https://doi.org/10.1038/s41537-019-0090-z

Prats, C., Arias, B., Moya-Higueras, J., et al. (2017). Evidence of an Epistatic Effect Between Dysbindin-1 and Neuritin-1 Genes on the Risk for Schizophrenia Spectrum Disorders. *European Psychiatry*, *40*, 60–64. https://doi.org/10.1016/j.eurpsy.2016.07.006

Pugliese, V., Bruni, A., Carbone, E. A., et al. (2019). Maternal stress, prenatal medical illnesses and obstetric complications: Risk factors for schizophrenia spectrum disorder, bipolar disorder and major depressive disorder. *Psychiatry Research*, *271*, 23–30. https://doi.org/10.1016/j.psychres.2018.11.023

Putz, U., Harwell, C., & Nedivi, E. (2005). Soluble CPG15 expressed during early development rescues cortical progenitors from apoptosis. *Nature Neuroscience*, *8*(3), 322–331. https://doi.org/10.1038/nn1407

Raben, T. G., Lello, L., Widen, E., et al. (2022). From Genotype to Phenotype: Polygenic Prediction of Complex Human Traits. *Methods in Molecular Biology (Clifton, N.J.)*, *2467*, 421–446. https://doi.org/10.1007/978-1-0716-2205-6_15

Rabinowitz, J., Levine, S. Z., & Häfner, H. (2006). A population based elaboration of the role of age of onset on the course of schizophrenia. *Schizophrenia Research*, *88*(1–3), 96–101. https://doi.org/10.1016/j.schres.2006.07.007

Radua, J., Ramella-Cravaro, V., Ioannidis, J. P. A., et al. (2018). What causes psychosis? An umbrella review of risk and protective factors. *World Psychiatry: Official Journal of the World Psychiatric Association (WPA)*, *17*(1), 49–66. https://doi.org/10.1002/wps.20490

Rajji, T. K., Ismail, Z., & Mulsant, B. H. (2009). Age at onset and cognition in schizophrenia: Meta-analysis. *The British Journal of Psychiatry*, *195*(4), 286–293. https://doi.org/10.1192/bjp.bp.108.060723

Ramsay, I. S., Nienow, T. M., Marggraf, M. P., et al. (2017). Neuroplastic changes in patients with schizophrenia undergoing cognitive remediation: Triple-blind trial. *The British Journal of Psychiatry*, *210*(3), 216–222. https://doi.org/10.1192/bjp.bp.115.171496

Rapoport, J. L., & Gogtay, N. (2011). Childhood onset schizophrenia: Support for a progressive neurodevelopmental disorder. *International Journal of Developmental Neuroscience*, *29*(3), 251–258. https://doi.org/10.1016/j.ijdevneu.2010.10.003

Ratliff, J. C., Palmese, L. B., Reutenauer, E. L., et al. (2012). The effect of dietary and physical activity pattern on metabolic profile in individuals with schizophrenia: A cross-sectional study. *Comprehensive Psychiatry*, *53*(7), 1028–1033. https://doi.org/10.1016/j.comppsych.2012.02.003

Redlich, R., Schneider, I., Kerkenberg, N., et al. (2020). The role of BDNF methylation and Val66Met in amygdala reactivity during emotion processing. *Human Brain Mapping*, *41*(3), 594–604. https://doi.org/10.1002/hbm.24825

Reeder, C., & Wykes, T. (2010). *Computerised interactive remediation of cognition–Interactive training for schizophrenia (CIRCUITS)*. London: Kings College. https://scholar.google.com/scholar_lookup?hl=en&publication_year=2010&author=C.+Reeder&author=T.+Wykes&title=Computerised+interactive+remediation+of+cognition%E2%80%94Interactive+training+for+schizophrenia+%28CIRCUITS%29

Rees, E., & Kirov, G. (2021). Copy number variation and neuropsychiatric illness. *Current Opinion in Genetics & Development*, *68*, 57–63. https://doi.org/10.1016/j.gde.2021.02.014

Reichard, J., & Zimmer-Bensch, G. (2021). The Epigenome in Neurodevelopmental Disorders. *Frontiers in Neuroscience*, *15*. https://doi.org/10.3389/fnins.2021.776809

Reichenberg, A., Caspi, A., Harrington, H., et al. (2010). Static and Dynamic Cognitive Deficits in Childhood Preceding Adult Schizophrenia: A 30-Year Study. *American Journal of Psychiatry*, *167*(2), 160–169. https://doi.org/10.1176/appi.ajp.2009.09040574

Reshetnikov, V. V., Kisaretova, P. E., Ershov, N. I., et al. (2020). Genes associated with cognitive performance in the Morris water maze: An RNA-seq study. *Scientific Reports*, *10*(1), 22078. https://doi.org/10.1038/s41598-020-78997-6

Reynolds, G. P. (2022). The neurochemical pathology of schizophrenia: Post-mortem studies from dopamine to parvalbumin. *Journal of Neural Transmission*, *129*(5), 643–647. https://doi.org/10.1007/s00702-021-02453-6

Richetto, J., & Meyer, U. (2021). Epigenetic Modifications in Schizophrenia and Related Disorders: Molecular Scars of Environmental Exposures and Source of Phenotypic Variability. *Biological Psychiatry*, *89*(3), 215–226. https://doi.org/10.1016/j.biopsych.2020.03.008

Riley, M. R., & Constantinidis, C. (2016). Role of Prefrontal Persistent Activity in Working Memory. *Frontiers in Systems Neuroscience*, *9*, 181. https://doi.org/10.3389/fnsys.2015.00181

Ripke, S., Neale, B. M., Corvin, A., et al. (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature*, *511*(7510), 421–427. https://doi.org/10.1038/nature13595

Rizig, M. A., McQuillin, A., Ng, A., et al. (2012). A gene expression and systems pathway analysis of the effects of clozapine compared to haloperidol in the mouse brain implicates susceptibility genes for schizophrenia. *Journal of Psychopharmacology*, *26*(9), 1218–1230. https://doi.org/10.1177/0269881112450780

Roberts, R., Barksdale, K., Roche, J., et al. (2015). Decreased synaptic and mitochondrial density in the postmortem anterior cingulate cortex in schizophrenia. *Schizophrenia Research*, *168*(0), 543–553. https://doi.org/10.1016/j.schres.2015.07.016

Rognli, E. B., & Bramness, J. G. (2015). Understanding the Relationship Between Amphetamines and Psychosis. *Current Addiction Reports*, *2*(4), 285–292. https://doi.org/10.1007/s40429-015-0077-4

Romero, C., Werme, J., Jansen, P. R., et al. (2022). Exploring the genetic overlap between twelve psychiatric disorders. *Nature Genetics*, *54*(12), 1795–1802. https://doi.org/10.1038/s41588-022-01245-2

Rubio-Abadal, E., Ochoa, S., Barajas, A., et al. (2015). Birth weight and obstetric complications determine age at onset in first episode of psychosis. *Journal of Psychiatric Research*, *65*, 108–114. https://doi.org/10.1016/j.jpsychires.2015.03.018

Ruderfer, D. M., Charney, A. W., Readhead, B., et al. (2016). Polygenic overlap between schizophrenia risk and antipsychotic response: A genomic medicine approach. *The Lancet. Psychiatry*, *3*(4), 350. https://doi.org/10.1016/S2215-0366(15)00553-2

Ruiz-Perez, D., Guan, H., Madhivanan, P., et al. (2020). So you think you can PLS-DA? *BMC Bioinformatics*, *21*(1), 2. https://doi.org/10.1186/s12859-019-3310-7

Ruzicka, W. B., Subburaju, S., & Benes, F. M. (2015). Circuit- and Diagnosis-Specific DNA Methylation Changes at γ-Aminobutyric Acid–Related Genes in Postmortem Human Hippocampus in Schizophrenia and Bipolar Disorder. *JAMA Psychiatry*, *72*(6), 541. https://doi.org/10.1001/jamapsychiatry.2015.49

Ruzicka, W. B., Subburaju, S., & Benes, F. M. (2017). Variability of DNA Methylation within Schizophrenia Risk Loci across Subregions of Human Hippocampus. *Genes*, *8*(5), 143. https://doi.org/10.3390/genes8050143

Sandstrom, A., Sahiti, Q., Pavlova, B., et al. (2019). Offspring of parents with schizophrenia, bipolar disorder, and depression: A review of familial high-risk and molecular genetics studies. *Psychiatric Genetics*, *29*(5), 160–169. https://doi.org/10.1097/YPG.0000000000000240

Sanosaka, T., Namihira, M., & Nakashima, K. (2009). Epigenetic mechanism in sequential differentiation of neural stem cells. *Epigenetics*, *4*(2), 89–92. https://doi.org/10.4161/epi.4.2.8233

Santoro, M. L., Ota, V., de Jong, S., et al. (2018). Polygenic risk score analyses of symptoms and treatment response in an antipsychotic-naive first episode of psychosis cohort. *Translational Psychiatry*, *8*, 174. https://doi.org/10.1038/s41398-018-0230-7

Saraceno, B., van Ommeren, M., Batniji, R., et al. (2007). Barriers to improvement of mental health services in low-income and middle-income countries. *Lancet (London, England)*, *370*(9593), 1164–1174. https://doi.org/10.1016/S0140-6736(07)61263-X

Sato, H., Fukutani, Y., Yamamoto, Y., et al. (2012). Thalamus-Derived Molecules Promote Survival and Dendritic Growth of Developing Cortical Neurons. *The Journal of Neuroscience*, *32*(44), 15388–15402. https://doi.org/10.1523/JNEUROSCI.0293-12.2012

Schmitt, A., Falkai, P., & Papiol, S. (2023). Neurodevelopmental disturbances in schizophrenia: Evidence from genetic and environmental factors. *Journal of Neural Transmission*, *130*(3), 195–205. https://doi.org/10.1007/s00702-022-02567-5

Sellgren, C. M., Gracias, J., Watmuff, B., et al. (2019). Increased synapse elimination by microglia in schizophrenia patient-derived models of synaptic pruning. *Nature Neuroscience*, *22*(3), 374–385. https://doi.org/10.1038/s41593-018-0334-7

Selvendra, A., Toh, W. L., Neill, E., et al. (2022). Age of onset by sex in schizophrenia: Proximal and distal characteristics. *Journal of Psychiatric Research*, *151*, 454–460. https://doi.org/10.1016/j.jpsychires.2022.05.010

Seng, C., Luo, W., & Földy, C. (2022). Circuit formation in the adult brain. *The European Journal of Neuroscience*, *56*(3), 4187. https://doi.org/10.1111/ejn.15742

Sengupta, S. M., MacDonald, K., Fathalli, F., et al. (2017). Polygenic Risk Score associated with specific symptom dimensions in first-episode psychosis. *Schizophrenia Research*, *184*, 116–121. https://doi.org/10.1016/j.schres.2016.11.039

Sharaf, A. Y., Ossman, L. H., & Lachine, O. A. (2012). A cross-sectional study of the relationships between illness insight, internalized stigma, and suicide risk in individuals with schizophrenia. *International Journal of Nursing Studies*, *49*(12), 1512–1520. https://doi.org/10.1016/j.ijnurstu.2012.08.006

Sheffield, J. M., Karcher, N. R., & Barch, D. M. (2018). Cognitive Deficits in Psychotic Disorders: A Lifespan Perspective. *Neuropsychology Review*, *28*(4), 509–533. https://doi.org/10.1007/s11065-018-9388-2

Shimada, T., Sugiura, H., & Yamagata, K. (2013). Neuritin: A therapeutic candidate for promoting axonal regeneration. *World Journal of Neurology*, *3*(4), 138–143. https://doi.org/10.5316/wjn.v3.i4.138

Siafis, S., Davis, J. M., & Leucht, S. (2021). Antipsychotic drugs: From 'major tranquilizers' to Neuroscience-based-Nomenclature. *Psychological Medicine*, *51*(3), 522–524. https://doi.org/10.1017/S0033291719003957

Silbereis, J. C., Pochareddy, S., Zhu, Y., et al. (2016). The Cellular and Molecular Landscapes of the Developing Human Central Nervous System. *Neuron*, *89*(2), 248–268. https://doi.org/10.1016/j.neuron.2015.12.008

Silva, M. J. D., McKenzie, K., Harpham, T., et al. (2005). Social capital and mental illness: A systematic review. *Journal of Epidemiology & Community Health*, *59*(8), 619–627. https://doi.org/10.1136/jech.2004.029678

Singh, T., Poterba, T., Curtis, D., et al. (2022). Rare coding variants in ten genes confer substantial risk for schizophrenia. *Nature*, *604*(7906), Article 7906. https://doi.org/10.1038/s41586-022-04556-w

Smigielski, L., Jagannath, V., Rössler, W., et al. (2020). Epigenetic mechanisms in schizophrenia and other psychotic disorders: A systematic review of empirical human findings. *Molecular Psychiatry*, *25*(8), Article 8. https://doi.org/10.1038/s41380-019-0601-3

Smith, S. M., Jenkinson, M., Woolrich, M. W., et al. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, *23 Suppl 1*, S208-219. https://doi.org/10.1016/j.neuroimage.2004.07.051

Solmi, M., Radua, J., Olivola, M., et al. (2022). Age at onset of mental disorders worldwide: Large-scale meta-analysis of 192 epidemiological studies. *Molecular Psychiatry*, *27*(1), 281–295. https://doi.org/10.1038/s41380-021-01161-7

Solmi, M., Seitidis, G., Mavridis, D., et al. (2023). Incidence, prevalence, and global burden of schizophrenia—Data, with critical appraisal, from the Global Burden of Disease (GBD) 2019. *Molecular Psychiatry*, *28*(12), 5319–5327. https://doi.org/10.1038/s41380-023-02138-4

Son, H., Banasr, M., Choi, M., et al. (2012). Neuritin produces antidepressant actions and blocks the neuronal and behavioral deficits caused by chronic stress. *Proceedings of the National Academy of Sciences*, *109*(28), 11378–11383. https://doi.org/10.1073/pnas.1201191109

Spear, L. P. (2013). Adolescent Neurodevelopment. *The Journal of Adolescent Health : Official Publication of the Society for Adolescent Medicine*, *52*(2 0 2), S7. https://doi.org/10.1016/j.jadohealth.2012.05.006

Stilo, S. A., & Murray, R. M. (2019). Non-Genetic Factors in Schizophrenia. *Current Psychiatry Reports*, *21*(10), 100. https://doi.org/10.1007/s11920-019-1091-3

Straub, R. E., MacLean, C. J., O'Neill, F. A., et al. (1995). A potential vulnerability locus for schizophrenia on chromosome 6p24–22: Evidence for genetic heterogeneity. *Nature Genetics*, *11*(3), 287–293. https://doi.org/10.1038/ng1195-287

Stuchlik, A. (2014). Dynamic learning and memory, synaptic plasticity and neurogenesis: An update. *Frontiers in Behavioral Neuroscience*, *8*. https://doi.org/10.3389/fnbeh.2014.00106

Sudre, G., Frederick, J., Sharp, W., et al. (2020). Mapping associations between polygenic risks for childhood neuropsychiatric disorders, symptoms of attention deficit hyperactivity disorder, cognition, and the brain. *Molecular Psychiatry*, *25*(10), Article 10. https://doi.org/10.1038/s41380-019-0350-3

Sullivan, P. F., Kendler, K. S., & Neale, M. C. (2003). Schizophrenia as a Complex Trait: Evidence From a Meta-analysis of Twin Studies. *Archives of General Psychiatry*, *60*(12), 1187. https://doi.org/10.1001/archpsyc.60.12.1187

Sullivan, P. F., Yao, S., & Hjerling-Leffler, J. (2024). Schizophrenia genomics: Genetic complexity and functional insights. *Nature Reviews Neuroscience*, *25*(9), 611–624. https://doi.org/10.1038/s41583-024-00837-7

Sun, M., Sun, Z., Wu, X., et al. (2016). Mammalian Brain Development is Accompanied by a Dramatic Increase in Bipolar DNA Methylation. *Scientific Reports*, *6*(1), 32298. https://doi.org/10.1038/srep32298

Sut, E., Akgül, Ö., & Bora, E. (2024). Minor physical anomalies in schizophrenia and first-degree relatives in comparison to healthy controls: A systematic review and meta-analysis. *European Neuropsychopharmacology*, *86*, 55–64. https://doi.org/10.1016/j.euroneuro.2024.05.007

Tanahashi, S., Yamamura, S., Nakagawa, M., et al. (2012). Clozapine, but not haloperidol, enhances glial d-serine and L-glutamate release in rat frontal cortex and primary cultured astrocytes. *British Journal of Pharmacology*, *165*(5), 1543. https://doi.org/10.1111/j.1476-5381.2011.01638.x

Tandon, R., Bruijnzeel, D., & Rankupalli, B. (2013). Does change in definition of psychotic symptoms in diagnosis of schizophrenia in DSM-5 affect caseness? *Asian Journal of Psychiatry*, *6*(4), 330–332. https://doi.org/10.1016/j.ajp.2013.05.011

ten Donkelaar, H. J., Copp, A. J., Bekker, M., et al. (2023). Neurulation and Neural Tube Defects. In H. J. ten Donkelaar, M. Lammens, & A. Hori (Eds.), *Clinical Neuroembryology: Development and Developmental Disorders of the Human Central Nervous System* (pp. 249–312). Springer International Publishing. https://doi.org/10.1007/978-3-031-26098-8_4

Tetzlaff, J. E., Huppenbauer, C. B., Tanzer, L., et al. (2006). Motoneuron injury and repair. *Journal of Molecular Neuroscience*, *28*(1), 53–64. https://doi.org/10.1385/JMN:28:1:53

Thomas, M. L., Duffy, J. R., Swerdlow, N., et al. (2021). Detecting the Inverted-U in fMRI Studies of Schizophrenia: A Comparison of Three Analysis Methods. *Journal of the International Neuropsychological Society : JINS*, *28*(3), 258. https://doi.org/10.1017/S1355617721000424

Thormodsen, R., Jensen, J., Holmèn, A., et al. (2011). Prefrontal hyperactivation during a working memory task in early-onset schizophrenia spectrum disorders: An fMRI study. *Psychiatry Research: Neuroimaging*, *194*(3), 257–262. https://doi.org/10.1016/j.pscychresns.2011.05.011

Tienari, P., Wynne, L. C., Sorri, A., et al. (2004). Genotype–environment interaction in schizophrenia-spectrum disorder: Long-term follow-up study of Finnish adoptees. *The British Journal of Psychiatry*, *184*(3), 216–222. https://doi.org/10.1192/bjp.184.3.216

Tilborg, E. van, Theije, C. G. M. de, Hal, M. van, et al. (2017). Origin and dynamics of oligodendrocytes in the developing brain: Implications for perinatal white matter injury. *Glia*, *66*(2), 221. https://doi.org/10.1002/glia.23256

Tiruchinapalli, D. M., Ehlers, M. D., & Keene, J. D. (2008). Activity-dependent expression of RNA binding protein HuD and its association with mRNAs in neurons. *RNA Biology*, *5*(3), 157–168. https://doi.org/10.4161/rna.5.3.6782

Tooley, U. A., Bassett, D. S., & Mackey, A. P. (2021). Environmental influences on the pace of brain development. *Nature Reviews Neuroscience*, *22*(6), 372–384. https://doi.org/10.1038/s41583-021-00457-5

Trotta, A., Murray, R. M., & Fisher, H. L. (2015). The impact of childhood adversity on the persistence of psychotic symptoms: A systematic review and meta-analysis. *Psychological Medicine*, *45*(12), 2481–2498. https://doi.org/10.1017/S0033291715000574

Trubetskoy, V., Pardiñas, A. F., Qi, T., et al. (2022). Mapping genomic loci implicates genes and synaptic biology in schizophrenia. *Nature*, *604*(7906), Article 7906. https://doi.org/10.1038/s41586-022-04434-5

Uno, Y., & Coyle, J. T. (2019). Glutamate hypothesis in schizophrenia. *Psychiatry and Clinical Neurosciences*, *73*(5), 204–215. https://doi.org/10.1111/pcn.12823

Valle, R. (2020). Schizophrenia in ICD-11: Comparison of ICD-10 and DSM-5. *Revista de Psiquiatría y Salud Mental (English Edition)*, *13*(2), 95–104. https://doi.org/10.1016/j.rpsmen.2020.01.002

van Erp, T. G. M., Hibar, D. P., Rasmussen, J. M., et al. (2016). Subcortical brain volume abnormalities in 2028 individuals with schizophrenia and 2540 healthy controls via the ENIGMA consortium. *Molecular Psychiatry*, *21*(4), 547–553. https://doi.org/10.1038/mp.2015.63

van Hooijdonk, C. F. M., van der Pluijm, M., Smith, C., et al. (2023). Striatal dopamine synthesis capacity and neuromelanin in the substantia nigra: A multimodal imaging study in schizophrenia and healthy controls. *Neuroscience Applied*, *2*, 101134. https://doi.org/10.1016/j.nsa.2023.101134

Van, L., Boot, E., & Bassett, A. S. (2017). Update on the 22q11.2 deletion syndrome and its relevance to schizophrenia. *Current Opinion in Psychiatry*, *30*(3), 191–196. https://doi.org/10.1097/YCO.0000000000000324

van Oel, C. J., Sitskoorn, M. M., Cremer, M. P. M., et al. (2002). School Performance as a Premorbid Marker for Schizophrenia: A Twin Study. *Schizophrenia Bulletin*, *28*(3), 401–414. https://doi.org/10.1093/oxfordjournals.schbul.a006949

van Os, J., Pries, L.-K., Delespaul, P., et al. (2020). Replicated evidence that endophenotypic expression of schizophrenia polygenic risk is greater in healthy siblings of patients compared to controls, suggesting gene-environment interaction. The EUGEI study. *Psychological Medicine*, *50*(11), 1884–1897. https://doi.org/10.1017/S003329171900196X

Varese, F., Smeets, F., Drukker, M., et al. (2012). Childhood Adversities Increase the Risk of Psychosis: A Meta-analysis of Patient-Control, Prospective- and Cross-sectional Cohort Studies. *Schizophrenia Bulletin*, *38*(4), 661–671. https://doi.org/10.1093/schbul/sbs050

Veerman, S. R. T., Schulte, P. F. J., Begemann, M. J. H., et al. (2014). Clozapine Augmented with Glutamate Modulators in Refractory Schizophrenia: A Review and Metaanalysis. *Pharmacopsychiatry*, *47*, 185–194. https://doi.org/10.1055/s-0034-1383656

Velásquez, E., Martins-de-Souza, D., Velásquez, I., et al. (2019). Quantitative Subcellular Proteomics of the Orbitofrontal Cortex of Schizophrenia Patients. *Journal of Proteome Research*, *18*(12), 4240–4253. https://doi.org/10.1021/acs.jproteome.9b00398

Verdoux, H., Lengronne, J., Liraud, F., et al. (2000). Medication adherence in psychosis: Predictors and impact on outcome. A 2-year follow-up of first-admitted subjects. *Acta Psychiatrica Scandinavica*, *102*(3), 203–210. https://doi.org/10.1034/j.1600-0447.2000.102003203.x

Viana, J., Hannon, E., Dempster, E., et al. (2016). Schizophrenia-associated methylomic variation: Molecular signatures of disease and polygenic risk burden across multiple brain regions. *Human Molecular Genetics*, *26*(1), 210. https://doi.org/10.1093/hmg/ddw373

Villéga, F., Fernandes, A., Jézéquel, J., et al. (2024). Ketamine alleviates NMDA receptor hypofunction through synaptic trapping. *Neuron*, *112*(19), 3311-3328.e9. https://doi.org/10.1016/j.neuron.2024.06.028

Voisey, J., Swagell, C. D., Hughes, I. P., et al. (2012). A Novel DRD2 Single-Nucleotide Polymorphism Associated with Schizophrenia Predicts Age of Onset: HapMap Tag-Single-Nucleotide Polymorphism Analysis. *Genetic Testing and Molecular Biomarkers*, *16*(2), 77–81. https://doi.org/10.1089/gtmb.2011.0085

Wagh, V. V., Vyas, P., Agrawal, S., et al. (2021). Peripheral Blood-Based Gene Expression Studies in Schizophrenia: A Systematic Review. *Frontiers in Genetics*, *12*. https://doi.org/10.3389/fgene.2021.736483

Wagner, J. R., Busche, S., Ge, B., et al. (2014). The relationship between DNA methylation, genetic and expression inter-individual variation in untransformed human fibroblasts. *Genome Biology*, *15*(2), R37. https://doi.org/10.1186/gb-2014-15-2-r37

Walton, E., Hass, J., Liu, J., et al. (2016). Correspondence of DNA Methylation Between Blood and Brain Tissue and Its Application to Schizophrenia Research. *Schizophrenia Bulletin*, *42*(2), 406–414. https://doi.org/10.1093/schbul/sbv074

Wan, K., Mao, F., Li, Q., et al. (2020). Neuritin-overexpressing transgenic mice demonstrate enhanced neuroregeneration capacity and improved spatial learning and memory recovery after ischemia-reperfusion injury. *Aging (Albany NY)*, *13*(2), 2681–2699. https://doi.org/10.18632/aging.202318

Wang, K.-S., Liu, X., Zhang, Q., et al. (2011). Genome-wide association analysis of age at onset in schizophrenia in a European-American sample. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *156*(6), 671–680. https://doi.org/10.1002/ajmg.b.31209

Wang, L., Owusu-Hammond, C., Sievert, D., et al. (2023). Stem Cell Based Organoid Models of Neurodevelopmental Disorders. *Biological Psychiatry*, *93*(7), 622. https://doi.org/10.1016/j.biopsych.2023.01.012

Wang, S., Sun, C., Walczak, C. A., et al. (1995). Evidence for a susceptibility locus for schizophrenia on chromosome 6pter–p22. *Nature Genetics*, *10*(1), 41–46. https://doi.org/10.1038/ng0595-41

Wang, Y., Chen, X., Liu, B., et al. (2020). Understanding the Relationship Between Human Brain Structure and Function by Predicting the Structural Connectivity From Functional Connectivity. *IEEE Access*, *8*, 1–1. https://doi.org/10.1109/ACCESS.2020.3039837

Ward, L. D., & Kellis, M. (2016). HaploReg v4: Systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. *Nucleic Acids Research*, *44*(Database issue), D877–D881. https://doi.org/10.1093/nar/gkv1340

Watanabe, Y., Nunokawa, A., & Someya, T. (2013). Association of the BDNF C270T polymorphism with schizophrenia: Updated meta-analysis. *Psychiatry and Clinical Neurosciences*, *67*(2), 123–125. https://doi.org/10.1111/pcn.12018

Watanabe, Y., Tanaka, H., Tsukabe, A., et al. (2014). Neuromelanin Magnetic Resonance Imaging Reveals Increased Dopaminergic Neuron Activity in the Substantia Nigra of Patients with Schizophrenia. *PLoS ONE*, *9*(8), e104619. https://doi.org/10.1371/journal.pone.0104619

Weiner, D. J., Nadig, A., Jagadeesh, K. A., et al. (2023). Polygenic architecture of rare coding variation across 394,783 exomes. *Nature*, *614*(7948), 492–499. https://doi.org/10.1038/s41586-022-05684-z

White, T., Hongwanishkul, D., & Schmidt, M. (2011). Increased anterior cingulate and temporal lobe activity during visuospatial working memory in children and adolescents with schizophrenia. *Schizophrenia Research*, *125*(2), 118–128. https://doi.org/10.1016/j.schres.2010.11.014

Wibrand, K., Messaoudi, E., Håvik, B., et al. (2006). Identification of genes co-upregulated with Arc during BDNF-induced long-term potentiation in adult rat dentate gyrus in vivo. *European Journal of Neuroscience*, *23*(6), 1501–1511. https://doi.org/10.1111/j.1460-9568.2006.04687.x

Williamson, J. M., & Lyons, D. A. (2018). Myelin Dynamics Throughout Life: An Ever-Changing Landscape? *Frontiers in Cellular Neuroscience*, *12*. https://doi.org/10.3389/fncel.2018.00424

Wiltink, S., Velthorst, E., Nelson, B., et al. (2015). Declining transition rates to psychosis: The contribution of potential changes in referral pathways to an ultra-high-risk service. *Early Intervention in Psychiatry*, *9*(3), 200–206. https://doi.org/10.1111/eip.12105

Wockner, L. F., Noble, E. P., Lawford, B. R., et al. (2014). Genome-wide DNA methylation analysis of human brain tissue from schizophrenia patients. *Translational Psychiatry*, *4*(1), e339–e339. https://doi.org/10.1038/tp.2013.111

Woo, H. J., Yu, C., Kumar, K., et al. (2017). Large-scale interaction effects reveal missing heritability in schizophrenia, bipolar disorder and posttraumatic stress disorder. *Translational Psychiatry*, *7*(4), Article 4. https://doi.org/10.1038/tp.2017.61

Woodberry, K. A., Giuliano, A. J., & Seidman, L. J. (2008). Premorbid IQ in Schizophrenia: A Meta-Analytic Review. *American Journal of Psychiatry*, *165*(5), 579–587. https://doi.org/10.1176/appi.ajp.2008.07081242

Woolston, A. L., Hsiao, P.-C., Kuo, P.-H., et al. (2017). Genetic loci associated with an earlier age at onset in multiplex schizophrenia. *Scientific Reports*, *7*(1), 6486. https://doi.org/10.1038/s41598-017-06795-8

Workman, A. D., Charvet, C. J., Clancy, B., et al. (2013). Modeling transformations of neurodevelopmental sequences across mammalian species. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *33*(17), 7368–7383. https://doi.org/10.1523/JNEUROSCI.5746-12.2013

Wu, C., Bendriem, R. M., Garamszegi, S. P., et al. (2017). RNA sequencing in post-mortem human brains of neuropsychiatric disorders. *Psychiatry and Clinical Neurosciences*, *71*(10), 663–672. https://doi.org/10.1111/pcn.12550

Wu, D., & Jiang, T. (2020). Schizophrenia-related abnormalities in the triple network: A meta-analysis of working memory studies. *Brain Imaging and Behavior*, *14*(4), 971–980. https://doi.org/10.1007/s11682-019-00071-1

Wu, Y.-T., Prina, A. M., & Brayne, C. (2015). The association between community environment and cognitive function: A systematic review. *Social Psychiatry and Psychiatric Epidemiology*, *50*(3), 351–362. https://doi.org/10.1007/s00127-014-0945-6

Wykes, T., Brammer, M., Mellers, J., et al. (2002). Effects on the brain of a psychological treatment: Cognitive remediation therapy: Functional magnetic resonance imaging in schizophrenia. *The British Journal of Psychiatry*, *181*(2), 144–152. https://doi.org/10.1192/bjp.181.2.144

Yang, J., Kang, C., Wu, C., et al. (2021). Pharmacogenetic associations of NRG1 polymorphisms with neurocognitive performance and clinical symptom response to risperidone in the untreated schizophrenia. *Schizophrenia Research*, *231*, 67–69. https://doi.org/10.1016/j.schres.2021.03.001

Yao, J.-J., Gao, X.-F., Chow, C.-W., et al. (2012). Neuritin Activates Insulin Receptor Pathway to Up-regulate Kv4.2-mediated Transient Outward K+ Current in Rat Cerebellar Granule Neurons *. *Journal of Biological Chemistry*, *287*(49), 41534–41545. https://doi.org/10.1074/jbc.M112.390260

Yoshimi, A., Suda, A., Hayano, F., et al. (2016). Effects of genotypes on orbitofrontal sulcogyral patterns in Japanese patients diagnosed with schizophrenia. *Psychiatry and Clinical Neurosciences*, *70*(7), 261–268. https://doi.org/10.1111/pcn.12384

Yu, L., Tasaki, S., Schneider, J. A., et al. (2020). Cortical Proteins Associated With Cognitive Resilience in Community-Dwelling Older Persons. *JAMA Psychiatry*, *77*(11), 1172–1180. https://doi.org/10.1001/jamapsychiatry.2020.1807

Yung, A. R., Yuen, H. P., McGorry, P. D., et al. (2005). Mapping the onset of psychosis: The Comprehensive Assessment of At-Risk Mental States. *The Australian and New Zealand Journal of Psychiatry*, *39*(11–12), 964–971. https://doi.org/10.1080/j.1440-1614.2005.01714.x

Zahari, Z., Teh, L. K., Ismail, R., et al. (2011). Influence of DRD2 polymorphisms on the clinical outcomes of patients with schizophrenia. *Psychiatric Genetics*, *21*(4), 183. https://doi.org/10.1097/YPG.0b013e3283437250

Zamorano, S., Sáez-Alonso, M., González-Sanguino, C., et al. (2023). Social Stigma Towards Mental Health Problems in Spain: A Systematic Review. *Clinical and Health*, *34*(1), 23–34. https://doi.org/10.5093/clysa2023a5

Zhan, N., Sham, P. C., So, H.-C., et al. (2023). The genetic basis of onset age in schizophrenia: Evidence and models. *Frontiers in Genetics*, *14*. https://doi.org/10.3389/fgene.2023.1163361

Zhang, A., Yu, J., Liu, J., et al. (2007). The DNA methylation profile within the 5'-regulatory region of DRD2 in discordant sib pairs with schizophrenia. *Schizophrenia Research*, *90*(1–3), 97–103. https://doi.org/10.1016/j.schres.2006.11.007

Zhang, L., Tie, X., Che, F., et al. (2023). Novel maternal duplication of 6p22.3-p25.3 with subtelomeric 6p25.3 deletion: New clinical findings and genotype–phenotype correlations. *Molecular Cytogenetics*, *16*(1), 11. https://doi.org/10.1186/s13039-023-00640-6

Zhao, H., Xu, J., Pang, L., et al. (2015). Genome-wide DNA methylome reveals the dysfunction of intronic microRNAs in major psychosis. *BMC Medical Genomics*, *8*(1), 62. https://doi.org/10.1186/s12920-015-0139-4

Zhao, Q.-R., Lu, J.-M., Li, Z.-Y., et al. (2018). Neuritin promotes neurite and spine growth in rat cerebellar granule cells via L-type calcium channel-mediated calcium influx. *Journal of Neurochemistry*, *147*(1), 40–57. https://doi.org/10.1111/jnc.14535

Zhou, S., & Zhou, J. (2014). Neuritin, A Neurotrophic Factor in Nervous System Physiology. *Current Medicinal Chemistry*, *21*(10), 1212–1219. https://doi.org/10.2174/09298673216661312180933 27

Zhou, Y., & Danbolt, N. C. (2014). Glutamate as a neurotransmitter in the healthy brain. *Journal of Neural Transmission*, *121*(8), 799. https://doi.org/10.1007/s00702-014-1180-8

Zhou, Y., Song, H., & Ming, G. (2024). Genetics of human brain development. *Nature Reviews Genetics*, *25*(1), 26–45. https://doi.org/10.1038/s41576-023-00626-5

Zhu, Y., Sousa, A. M. M., Gao, T., et al. (2018). Spatio-temporal transcriptomic divergence across human and macaque brain development. *Science (New York, N.Y.)*, *362*(6420), eaat8077. https://doi.org/10.1126/science.aat8077

Zimmer-Bensch, G. (2018). Diverse facets of cortical interneuron migration regulation – Implications of neuronal activity and epigenetics. *Brain Research*, *1700*, 160–169. https://doi.org/10.1016/j.brainres.2018.09.001

Zito, A., Cartelli, D., Cappelletti, G., et al. (2014). Neuritin 1 promotes neuronal migration. *Brain Structure and Function*, *219*(1), 105–118. https://doi.org/10.1007/s00429-012-0487-1

Zorumski, C. F., Izumi, Y., & Mennerick, S. (2016). Ketamine: NMDA Receptors and Beyond. *The Journal of Neuroscience*, *36*(44), 11158. https://doi.org/10.1523/JNEUROSCI.1547-16.2016

Zuk, O., Hechter, E., Sunyaev, S. R., et al. (2012). The mystery of missing heritability: Genetic interactions create phantom heritability. *Proceedings of the National Academy of Sciences*, *109*(4), 1193–1198. https://doi.org/10.1073/pnas.1119675109

9. Curriculum Vitae

CARMEN ALMODÓVAR PAYÁ
carmenalmodovarpaya@gmail.com
+34 675204110

Research Biologist
Neurobiology and Genetics of Psychotic Disorders, FIDMAG Research Foundation

ACADEMIC TRAINING

Pres. – 2019	**Doctoral thesis in biomedicine.** University of Barcelona.
2017 – 2016	**Master's degree in biological anthropology: specialization in human biodiversity and biomedical applications.** University of Barcelona (UB) and Autonomous University of Barcelona (UAB).
2014 – 2009	**Bachelor's degree in biomedical sciences.** University of Barcelona (UB).

WORK EXPERIENCE

Present Jan. 2023	**PhD researcher, La Marató de TV3 contract.** Neurobiology and Genetics of Psychotic disorders (NeuroBioGen), FIDMAG Germanes Hospitalàries Research Foundation.
Present Jan. 2023	**Assistant Professor.** Department of Evolutionary Biology, Ecology and Environmental Sciences. University of Barcelona (UB).
Dec.2022 Dec. 2019	**PhD researcher, CIBERSAM contract.** Neurobiology and Genetics of Psychotic disorders (NeuroBioGen), FIDMAG Germanes Hospitalàries Research Foundation.
May 2021 March 2021	**Assistant Professor.** Department of Evolutionary Biology, Ecology and Environmental Sciences. University of Barcelona (UB).
Dec. 2019 May 2017	**Research support technician, NARSAD contract.** Neurobiology and Genetics of Psychotic disorders (NeuroBioGen), FIDMAG Germanes Hospitalàries Research Foundation.

RESEARCH STAYS AND COLLABORATIONS

Dec. 2022 Sept. 2022	**PhD research stay.** Department of Neuropsychiatry and Laboratory of Molecular Psychiatry, Charité-Universitätsmedizin Berlin, Germany.
May 2018 Sep. 2017	**Extracurricular collaboration.** Neurobiology and Genetics of Psychotic Disorders (NeuroBioGen) research line, FIDMAG Sisters Hospitaliers Research Foundation.

RESEARCH GRANTS

Dec. 2022 – Sept. 2022 **Mobility Grant.** Grants for the realization of training stays in Spain and abroad financed by Banco Santander.

PARTICIPATION IN PROJECTS FUNDED VIA COMPETITIVE CALLS

2027 - 2024 **Researcher.** Identificación de biomarcadores genómico-fenotípicos de la psicosis: análisis de la descendencia de padres afectados y sus pedigríes de la cohorte PAFIP-FAMILIES (**PI23/01262**). Instituto de Salud Carlos III, Madrid, Spain. PI: Mar Fatjó-Vilas. 128,750.00€.

2026 – 2023 **Researcher.** Targeting mRNAs condensates in neurites for a better understanding of synaptic plasticity dysfunction in schizophrenia (**41/C/2022**). Fundació La Marató de TV3, Barcelona, Spain. PI: Mar Fatjó-Vilas. 299,906.25€.

2025 – 2023 **Researcher.** (Grupo CIBERSAM). Medicina Personalizada (MedPer) en la detección precoz del deterioro cognitivo (DC) preclínico. Desarrollo de un modelo predictivo de riesgo (**PMP22/00084**). Ministerio de Ciencia e Innovación, Madrid, Spain. PI: Ángeles Almeida Parra, PI Coordinated Project: Mar Fatjó-Vilas. 1,650,550€ (coordinated project: 171,985€).

2024 – 2022 **Researcher.** Grup de recerca consolidat FIDMAG Germanes Hospitalàries Research Foundation (**2022SGR1475**). Comissionat per a Universitat i Recerca del DIUE (Agència de Gestió d'Aguts Universitaris i de Recerca – AGAUR). PI: Edith Pomarol-Clotet. 40,000€.

2023 – 2021 **Researcher.** Neurodevelopment markers and schizophrenia: analysis of their shared genetic underpinnings and the modulation effect of prenatal stress (**PI20/01002**). Instituto de Salud Carlos III, Madrid, Spain. PI: Mar Fatjó-Vilas. 63,500€.

2021 – 2019 **Researcher.** Analysis of the epigenetic profile of the *NRN1* gene and its association with brain activity in schizophrenia. Acadèmia de les Ciències Mèdiques i de la Salut de Catalunya i Balears. PI: Edith Pomarol-Clotet and Mar Fatjó-Vilas. 30,000€.

SCIENTIFIC PUBLICATIONS

I am co-author of **13 published scientific papers in international** high-impact research journals, 5 of which as first author. Four of these publications are directly related to my doctoral thesis project (marked with ++). Moreover, I have **2 publications in national journals**, both as first author.

Articles in international scientific journals:

Submitted

- Almodóvar-Payá C, Moreno M, Guardiola-Ripoll M, Latorre-Guardia M, Morentin B, Garcia-Ruíz B, Pomarol-Clotet E, Callado LF, Gallego C, Fatjó-Vilas M. *Clozapine-related brain NRN1 expression patterns are associated with methylation and genetic variants in schizophrenia*. Submitted for peer review in an indexed journal ++

2024

1. Almodóvar-Payá C, París-Gómez I, Latorre-Guardia M, Guardiola-Ripoll M, Catalán R, Arias B, Penadés R, Fatjó-Vilas M. *NRN1 genetic variability and methylation changes as biomarkers for cognitive remediation therapy response in schizophrenia*. Prog Neuropsychopharmacol Biol Psychiatry. 2024 Oct 18;136:111175. doi: 10.1016/j.pnpbp.2024.111175. Epub ahead of print. PMID: 39426559. IF 2023=5.3, 35/354 (DI) Pharmacology & Pharmacy ++
2. Guardiola-Ripoll M, Sotero-Moreno A, Chaumette B, Kebir O, Hostalet N, Almodóvar-Payá C, Moreira M, Giralt-López M, Krebs MO, Fatjó-Vilas M. *Genetic and Neurodevelopmental Markers in Schizophrenia-Spectrum Disorders: Analysis of the Combined Role of the CNR1 Gene and Dermatoglyphics*. Biomedicines. 2024 Oct 7;12(10):2270. doi: 10.3390/biomedicines12102270. PMID: 39457583. IF 2023=3.9, 85/354 (Q1) Pharmacology & Pharmacy
3. Almodóvar-Payá C, Guardiola-Ripoll M, Giralt-López M, Oscoz-Irurozqui M, Canales-Rodríguez EJ, Madre M, Soler-Vidal J, Ramiro N, Callado LF, Arias B, Gallego C, Pomarol-Clotet E, Fatjó-Vilas M. *NRN1 epistasis with BDNF and CACNA1C: mediation effects on symptom severity through neuroanatomical changes in schizophrenia*. Brain Struct Funct. 2024 Jun;229(5):1299-1315. doi: 10.1007/s00429-024-02793-5. IF 2023=3.1, 3/23 (D1) Anatomy. ++
4. Penadés R, Almodóvar-Payá C, García-Rizo C, Ruíz V, Catalán R, Valero S, Wykes T, Fatjó-Vilas M, Arias B. *Changes in BDNF methylation patterns after cognitive remediation therapy in schizophrenia: A randomized and controlled trial*. J Psychiatr Res. 2024 May; 173:166-174. doi: 10.1016/j.jpsychires.2024.03.014. PMID: 38537483. IF 2023=4.8, 39/144 (Q2) Psychiatry.

2023

5. Guardiola-Ripoll M, Almodóvar-Payá C, Arias-Magnasco A, Latorre-Guardia M, Papiol S, Canales-Rodríguez EJ, García-León MA, Fuentes-Claramonte P, Salavert J, Tristany J, Torres L, Rodríguez-Cano E, Salvador R, Pomarol-Clotet E, Fatjó-Vilas M. *Human-specific evolutionary markers linked to foetal neurodevelopment modulate brain surface area in schizophrenia*. Commun Biol. 2023 Oct 13;6(1):1040. doi:

10.1038/s42003-023-05356-2. PMID: 37833414. **IF 2022=5.9, 12/92 (Q1) Biology.**

6. Salvador R, García-León MÁ, Feria-Raposo I, Botillo-Martín C, Martín-Lorenzo C, Corte-Souto C, Aguilar-Valero T, Gil-Sanz D, Porta-Pelayo D, Martín-Carrasco M, Del Olmo-Romero F, Maria Santiago-Bautista J, Herrero-Muñecas P, Castillo-Oramas E, Larrubia-Romero J, Rios-Alvarado Z, Antonio Larraz-Romeo J, Guardiola-Ripoll M, **Almodóvar-Payá C**, Fatjó-Vilas Mestre M, Sarró S, McKenna PJ, HHFingerprints Group, Pomarol-Clotet E. *Fingerprints as Predictors of Schizophrenia: A Deep Learning Study.* **Schizophr Bull**. 2023 May 3;49(3):738-745. doi: 10.1093/schbul/sbac173. PMID: 36444899. **IF 2022=6.6, 32/155 (Q1) Psychiatry.**

7. Oscoz-Irurozqui M, **Almodóvar-Payá C**, Guardiola-Ripoll M, Guerrero-Pedraza A, Hostalet N, Salvador R, Carrión MI, Maristany T, Pomarol-Clotet E, Fatjó-Vilas M. *Cannabis Use and Endocannabinoid Receptor Genes: A Pilot Study on Their Interaction on Brain Activity in First-Episode Psychosis.* **Int J Mol Sci**. 2023 Apr 19;24(8):7501. doi: 10.3390/ijms24087501. PMID: 37108689. **IF 2022=5.6, 66/285 (Q1) Biochemistry & Molecular Biology.**

2022

8. **Almodóvar-Payá C,** Guardiola-Ripoll M, Giralt-López M, Gallego C, Salgado-Pineda P, Miret S, Salvador R, Muñoz MJ, Lázaro L, Guerrero-Pedraza A, Parellada M, Carrión MI, Cuesta MJ, Maristany T, Sarró S, Fañanás L, Callado LF, Arias B, Pomarol-Clotet E, Fatjó-Vilas M. *NRN1 Gene as a Potential Marker of Early-Onset Schizophrenia: Evidence from Genetic and Neuroimaging Approaches.* **Int J Mol Sci**. 2022 Jul 5;23(13):7456. doi: 10.3390/ijms23137456. PMID: 35806464. **IF 2022=5.6, 66/285 (Q1) Biochemistry & Molecular Biology.** ++

9. Guardiola-Ripoll M, Sotero-Moreno A, **Almodóvar-Payá C**, Hostalet N, Guerrero-Pedraza A, Ramiro N, Ortiz-Gil J, Arias B, Madre M, Soler-Vidal J, Salvador R, McKenna PJ, Pomarol-Clotet E, Fatjó-Vilas M. *Combining fMRI and DISC1 gene haplotypes to understand working memory-related brain activity in schizophrenia*. **Sci Rep**. 2022 May 5;12(1):7351. doi: 10.1038/s41598-022-10660-8. PMID: 35513527. **IF 2022=4.6, 22/73 (Q2) Multidisciplinary Sciences.**

10. Guardiola-Ripoll M, **Almodóvar-Payá C**, Lubeiro A, Salvador R, Salgado-Pineda P, Gomar JJ, Guerrero-Pedraza A, Sarró S, Maristany T, Fernández-Linsenbarth I, Hernández-García M, Papiol S, Molina V, Pomarol-Clotet E, Fatjó-Vilas M. *New insights of the role of the KCNH2 gene in schizophrenia: An fMRI case-control study.* **Eur Neuropsychopharmacol**. 2022 Jul;60:38-47. doi:

10.1016/j.euroneuro.2022.04.012. Epub 2022 May 26. PMID: 35635995. **IF 2022=5.6, 35/212 (Q1) Clinical Neurology.**

11. Guardiola-Ripoll M, **Almodóvar-Payá C**, Lubeiro A, Sotero A, Salvador R, Fuentes-Claramonte P, Salgado-Pineda P, Papiol S, Ortiz-Gil J, Gomar JJ, Guerrero-Pedraza A, Sarró S, Maristany T, Molina V, Pomarol-Clotet E, Fatjó-Vilas M. *A functional neuroimaging association study on the interplay between two schizophrenia genome-wide associated genes (CACNA1C and ZNF804A).* **Eur Arch Psychiatry Clin Neurosci**. 2022 Oct;272(7):1229-1239. doi: 10.1007/s00406-022-01447-z. Epub 2022 Jul 7. PMID: 35796825. **IF 2022=4.7, 52/212 (Q1) Clinical Neurology.**

2021

12. Santo-Angles A, Fuentes-Claramonte P, Argila-Plaza I, Guardiola-Ripoll M, **Almodóvar-Payá C**, Munuera J, McKenna PJ, Pomarol-Clotet E, Radua J. *Reward and fictive prediction error signals in ventral striatum: asymmetry between factual and counterfactual processing.* **Brain Struct Funct**. 2021 Jun;226(5):1553-1569. doi: 10.1007/s00429-021-02270-3. Epub 2021 Apr 11. PMID: 33839955. **IF 2021=3.748, 1/21 (D1) Anatomy & Morphology.**

13. Fuentes-Claramonte P, Santo-Angles A, Argila-Plaza I, Lechón M, Guardiola-Ripoll M, **Almodóvar-Payá C**, Cullen B, Evans JJ, Manly T, Gee A, Maristany T, Sarró S, Pomarol-Clotet E, McKenna PJ, Salvador R. *Brain imaging of executive function with the computerised multiple elements test.* **Brain Imaging Behav**. 2021 Oct;15(5):2317-2329. doi: 10.1007/s11682-020-00425-0. Epub 2021 Jan 26. PMID: 33501628. **IF 2021=3.224, 7/14 (Q2) Neuroimaging.**

2020

14. Fullana MA, Tortella-Feliu M, Fernández de la Cruz L, Chamorro J, Pérez-Vigil A, Ioannidis JPA, Solanes A, Guardiola M, **Almodóvar C**, Miranda-Olivos R, Ramella-Cravaro V, Vilar A, Reichenberg A, Mataix-Cols D, Vieta E, Fusar-Poli P, Fatjó-Vilas M, Radua J. *Risk and protective factors for anxiety and obsessive-compulsive disorders: an umbrella review of systematic reviews and meta-analyses.* **Psychol Med**. 2020 Jun;50(8):1300-1315. doi: 10.1017/S0033291719001247. Epub 2019 Jun 7. PMID: 31172897. **IF 2020=7.723, 15/156 (D1) Psychiatry.**

15. Lubeiro A, Fatjó-Vilas M, Guardiola M, **Almodóvar C**, Gomez-Pilar J, Cea-Cañas B, Poza J, Palomino A, Gómez-García M, Zugasti J, Molina V. *Analysis of KCNH2 and CACNA1C schizophrenia risk genes on EEG functional network modulation during an auditory odd-ball task.* **Eur Arch Psychiatry Clin Neurosci**. 2020 Jun;270(4):433-442. doi: 10.1007/s00406-018-0977-0. Epub 2019 Jan 3. PMID: 30607529. **IF 2020=5.276, 41/208 (Q1) Clinical Neurology**

Articles in national scientific journals:

2020 1. Fatjó-Vilas M, **Almodóvar-Payá C,** Guardiola-Ripoll M, Oscoz-Irurozqui M, Sotero A, Salgado-Pineda P, Salvador R, McKenna PJ, Pomarol-Clotet E. Factors genètics i salut mental: estudis aplicats a la recerca de les causes i de la millora diagnòstica. **The Business, Research, Ageing, Innovation & Neuroscience journal (Brain).** 2020 September; 4:22-24.

2018 2. **Almodóvar-Payá C**, Salgado-Pineda P, Gallego C, Arias B, Pomarol-Clotet E, McKenna PJ, Fatjó-Vilas M. *NRN1* gene and brian structure: an association study in patients with schizophrenia and healthy subjects. **Libro de Actas del XX Congreso Nacional de Antropología Física: La Antropología Física en la Era de la Genómica.** 2018 April.

INTERNATIONAL & NATIONAL CONFERENCES COMMUNICATIONS

I participated as co-author of **41 scientific communications at international conferences**, 13 of which as the first presenting author (12 posters and 2 oral communications). Thirteen of these communications are directly related to my doctoral thesis project (marked with ++). Moreover, I have received as a co-author **2 awards for the best poster communication** (marked with **).

International:

2024 1. **Almodóvar-Payá C,** Moreno M, Latorre-Guardia M, Pomarol-Clotet E, Callado LF, Gallego C, Fatjó-Vilas M. Unveiling the *NRN1* gene epigenetic-expression brain correlates in schizophrenia. *2024 Congress of the Schizophrenia International Research Society (SIRS).* **Poster communication.** April 3thr-7th 2024, Florence, Italy. ++

2. Riquelme G, Guardiola-Ripoll M, Almodóvar-Payá C, Latorre-Guardia M, Pomarol-Clotet E, Ramos B, Fatjó-Vilas M. Unravelling the Polygenic Burden of Transcription Factors in Schizophrenia: The Importance of the Prenatal Transcriptome. **Poster communication.** *2024 Congress of the Schizophrenia International Research Society (SIRS)*. April 3thr-7th 2024, Florence, Italy.

3. Penadés R, **Almodóvar-Payá C**, García-Rizo C, Ruíz V, Catalán R, Valero S, Wykes T, Fatjó-Vilas M, Arias B. Improvement in cognition during cogntive remediation is related with epigenetic changes in BDNF gene. **Poster communication.** *Congress of the Schizophrenia International Research Society (SIRS)*. April 3thr-7th 2024, Florence, Italy.

4. Latorre-Guardia M, **Almodóvar-Payá C**, Arias B, Guardiola-Ripoll M, Fuentes-Claramonte P, García-León MA, Pomarol-Clotet E, Fatjó-Vilas M. Metilación y estructura cerebral en esquizofrenia: análisis del *gen FKBP5*. **Oral communication**. *ACCAP 2024: Psiquiatría de Enlace: Intersección entre la salud física y mental*. March 20th-23thr 2024, virtual.

2023

5. Almodóvar-Payá C, Guardiola-Ripoll M, Gallego C, Moreno M, Latorre-Guardia M, Canales-Rodríguez EJ, Callado LF, Miret S, Arias B, Penadés R, Salvador R, Pomarol-Clotet E, Fatjó-Vilas M. Molecular and neuroimaging analyses of *NRN1'S* impact on schizophrenia. Poster communication. **Poster communication**. *11th International Brain Research Organization (IBRO) World Congress of Neuroscience*. September 9th-13th 2023, Granada, Spain. ++

6. Almodóvar-Payá C, Guardiola-Ripoll M, Carme G, Guerrero-Pedraza A, Torres ML,Salvador R, Pomarol-Clotet E, Fatjó-Vilas M. Epistasis effect between *NRN1* and *BDNF* on brain structure mediates symptom severity in schizophrenia. **Poster communication**. *36th European College of Neuropsychopharmacology Congress (ECNP) Congress*. October 7th-10th 2023, Barcelona, Spain. ++

7. Guardiola-Ripoll M, **Almodóvar-Payá C**, Arias-Magnasco A, Latorre-Guardia M, Papiol S, Canales-Rodríguez EJ, García-León MA, Salvador R, Pomarol-Clotet E, Fatjó-Vilas M. The cortical architecture in schizophrenia is influenced by genetic variability in human-specific evolutionary markers guiding the early neurodevelopmental transcriptional machinery. **Poster communication**. *36th European College of Neuropsychopharmacology Congress (ECNP) Congress*. October 7th-10th 2023, Barcelona, Spain.

8. Gómez I, **Almodóvar-Payá C**, Latorre-Guardia M, Arias B, Penadès R, Fatjó-Vilas M. Association of methylation changes in *NRN1* gene and cognitive performance after cognitive remediation therapy in schizophrenia. **Poster communication**. *36th European College of Neuropsychopharmacology Congress (ECNP) Congress*. October 7th-10th 2023, Barcelona, Spain. ++

9. Guardiola-Ripoll M, **Almodóvar-Payá C**, Arias A, Latorre-Guardia M, Canales-Rodríguez EJ, García-León MA, Fuentes-Claramonte P, Salvador R, Pomarol-Clotet E, Papiol S, Fatjó-Vilas M. Genetic markers of evolution and brain cortical surface area in schizophrenia: are they related? **Poster communication**. *Organization for Human Brain Mapping (OHBM) 2023 Congress*. July 22nd-26th 2023, Montréal, Canada.

2022

10. Almodóvar-Payá C, Guardiola-Ripoll M, Gallego C, Callado LF, Giralt-Lopez M, Latorre-Guardia M, Salgado-Pineda P, Canales-Rodríguez E,

Miret S, Fañanás L, Penadés R, Fuentes-Claramonte P, Salvador R, Arias B, Pomarol-Clotet E, Fatjó-Vilas M. The effect of *NRN1* on schizophrenia: an intersectional study integrating molecular and neuroimaging approaches. **Poster communication**. *35th European College of Neuropsychopharmacology Congress (ECNP) Congress*. October 15th-18th 2022, Vienna, Austria. ++

11. Martínez M, Guardiola-Ripoll M, Oscoz-Irurozqui M, **Almodóvar-Payá C**, Guerrero-Pedraza A, Hostalet N, Salvador R, Carrión MI, Maristany T, Pomarol-Clotet E, Fatjó-Vilas M. *DRD2* and *DRD3* genes, cannabis use and brain activity in first-episode psychosis. **Poster Jam**. *35th European College of Neuropsychopharmacology Congress (ECNP) Congress*. October 15th-18th 2022, Vienna, Austria.

12. Martínez M, Guardiola-Ripoll M, Oscoz-Irurozqui M, **Almodóvar-Payá C**, Guerrero-Pedraza A, Hostalet N, Salvador R, Carrión MI, Maristany T, Pomarol-Clotet E, Fatjó-Vilas M. Polymorphic variants at dopamine receptor genes (DRD2 and DRD3) and cannabis use: effects on brain activity in first-episode psychosis (FEP). **Poster Communication**. *22nd World Congress of Psychiatry (WCP)*. August 3rd-6th 2022, Bangkok, Thailand.

13. Sotero-Moreno, A; Guardiola-Ripoll, M; Moreira, M; Giralt-López, M; Almodóvar-Payá, C; Hostalet, N; Miret, S; Campanera, S; Muñoz, MJ; Salvador, R; Fañanás, L; Pomarol-Clotet, E; Fatjó-Vilas, M. Biomarcadores del neurodesarrollo en esquizofrenia: genética, neuroimagen y dermatoglifos. **Oral communication**. *I Congreso Multidisciplinario Internacional del Hospital Psiquiátrico Santa Rosita. Salud Mental a través del Tiempo, por un Hospital Psiquiátrico ¡SIN ESTIGMAS!*. May 18th-20th 2022, virtual.

14. **Almodóvar-Payá C**, Guardiola-Ripoll M, Gallego C, Callado LF, Moreno M, Latorre-Guardia M, Garcia-Torrents E, Canales-Rodriguez E, Giralt M, Miret S, Arias B, Penadés R, Salvador R, Pomarol-Clotet E, Fatjó-Vilas M. Assessing the impact of *NRN1* on clinical hallmarks of schizophrenia by combining molecular and neuroimaging analyses. **Poster Communication.** *2022 Congress of the Schizophrenia International Research Society (SIRS)*. April 6th-10th 2022, Florence, Italy. ++

15. Latorre-Guardia M, **Almodóvar-Payá C**, Arias B, Penadés B, Guardiola-Ripoll M, García-León MA, Fuentes-Claramonte P, Pomarol-Clotet E, Fatjó-Vilas M. *FKBP5* methylation patterns and brain structural correlates in schizophrenia. **Oral communication**. *2022 Congress of the Schizophrenia International Research Society (SIRS)*. April 6th-10th 2022, Florence, Italy.

16. Guardiola-Ripoll M, Sotero-Moreno A, Kebir O, Chaumette B, **Almodóvar-Payá C**, Hostalet N, Moreira M, Giralt M, Salvador R, Odile-Krebs M, Fatjó-Vilas M. Markers in Schizophrenia-Spectrum disorders: Analysis of the Combined Role of the Cannabinoid Receptor 1 Gene (*CNR1*) and Dermatoglyphics. **Poster Communication**. *2022 Congress of the Schizophrenia International Research Society (SIRS)*. April 6th-10th 2022, Florence, Italy.

2021

17. **Almodóvar-Payá C**, Latorre-Guardia M, Arias B, Penadés R, Guardiola-Ripoll M, Fuentes-Claramonte P, García-León MA, Pomarol-Clotet E, Fatjó-Vilas M. Methylation and brain structure in schizophrenia: analysis of the role of *COMT* and *BDNF* genes. **Poster communication**. *World Congress of Psychiatric Genetics (WCPG)*. October 11th-15th 2021, virtual. ++

18. **Almodóvar-Payá C,** Guardiola-Ripoll M, Gallego C, Hostalet N, Sotero A, Guerreo-Pedraza A, Ramiro N, Torres L, Salvador R, Pomarol-Clotet E, Fatjó-Vilas M. Analysis of epistatic effects between schizophrenia-related genes and their role in clinical outcomes and brain activity. **Poster communication**. *World Congress of Psychiatric Genetics (WCPG)*. October 11th-15th 2021, virtual. ++

19. Oscoz-Irurozqui M, **Almodóvar-Payá C**, Guardiola-Ripoll M, Guerreo-Pedraza, Hostalet N, Pomarol-Clotet E, Fatjó-Vilas M. Cannabis Use and *CNR1* Gene: Effects on Psychotic Symptoms and Cognition in First-episode Psychosis. **Poster communication**. *World Congress of Psychiatric Genetics (WCPG)*. October 11th-15th 2021, virtual.

20. Moreno M, Ortiz R, **Almodóvar-Payá C**, Guardiola-Ripoll M, Callado LF, Fatjó-Vilas M, Gallego C. Study of *NRN1* expression in brain: analysis in individuals diagnosed with schizophrenia and healthy subjects. **Poster communication**. *World Congress of Psychiatric Genetics (WCPG)*. October 11th-15th 2021, virtual.

21. **Almodóvar-Payá C**, Latorre-Guardia M, Arias B, Penadés R, Guardiola-Ripoll M, Fuentes-Claramonte P, García-León M, Pomarol-Clotet E, Fatjó-VilasM. Candidate genes of schizophrenia methylation patterns and brain structural correlates. **Poster Communication**. *34th European College of Neuropsychopharmacology Congress (ECNP)*. October 2nd-5th 2021, Lisbon, Portugal. ++

22. Oscoz Irurozqui M, **Almodóvar-Payá C**, Guardiola-Ripoll M, Guerrero-Pedraza A, Aquino A, Salgado-Pineda P, Sarró S, Pomarol-Clotet E, Fatjó-Vilas M. Cannabinoid receptor genes, cannabis use and brain activity in first-episode psychosis. **Poster Communication**. *34th*

European College of Neuropsychopharmacology Congress (ECNP). October 2nd-5th 2021, Lisbon, Portugal.

23. Almodóvar-Payá C, Guardiola-Ripoll M, Gallego C, Hostalet N, Sotero A, Guerrero-Pedraza A, Ramiro N, Torres Ll, Salvador R, Pomarol-Clotet E, Fatjó-Vilas M. Evidence of epistatic effect involving the *NRN1*, *KCNH2* and *CACNA1C* genes on the risk for schizophrenia-spectrum disorders and its impact on psychopathology. Poster communication. *2021 Congress of the Schizophrenia International Research Society (SIRS)*. April 17th-21st 2021, virtual. ++

24. Almodóvar-Payá C, García-Torrents E, Guardiola-Ripoll M, Canales-Rodríguez E, Hostalet N, Gallego C, Martín-Subero M, Madre M, Pomarol-Clotet E, Fatjó-Vilas M. The effect of *NRN1* gene on cortical thickness in healthy subjects and subjects with schizophrenia. Poster communication. 2021 *Congress of the Schizophrenia International Research Society (SIRS)*. April 17th-21st 2021, virtual. ++

25. Oscoz-Irurozqui M, Almodóvar-Payá C, Guardiola-Ripoll M, Guerrero-Pedraza A, Salgado-Pineda P, Sarró S, Pomarol-Clotet E, Fatjó-Vilas M. The interplay between cannabinoid receptor genes and cannabis use: effects on brain activity in first-episode psychosis. Poster communication. *2021 Congress of the Schizophrenia International Research Society (SIRS)*. April 17th-21st 2021, virtual.

26. Guardiola-Ripoll M, Almodóvar-Payá C, Lubeiro A, Sotero A, Salgado-Pineda P, Salvador R, Papiol S, Ortiz-Gil J, Sarró S, McKenna PJ, Maristany T, Molina V, Pomarol-Clotet E, Fatjó-Vilas M. The interplay between two schizophrenia GWAS genes on fMRI working memory response: Evidence of *CACNA1C* x *ZNF804A* epistatic effect on brain function. Poster communication. *2021 Congress of the Schizophrenia International Research Society (SIRS)*. April 17th-21st 2021, virtual.

27. Guardiola-Ripoll M, Sotero A, Almodóvar-Payá C, Hostalet N, Salgado-Pineda P, Salvador R, Ortiz-Gil J, Sarró S, McKenna PJ, Madre M, Pomarol-Clotet E, Fatjó-Vilas M. DISC1 gene and brain activity: a genetic neuroimaging association study. Oral communication. *2021 Congress of the Schizophrenia International Research Society (SIRS)*. April 17th-21st 2021, virtual.

28. Almodóvar-Payá C, Garcia-Torrents E, Guardiola-Ripoll M, Canales-Rodríguez EJ, Hostalet N, Gallego C, Martín-Subero M, Madre M, Pomarol-Clotet E, Fatjó-Vilas M. The role of NRN1 gene in schizophrenia: analysis of its genetic variability in cortical thickness.

Oral communication. *XX World Psychiatry Association (WPA) World Congress of Psychiatry.* May 10th-13th 2021, virtual. ++

29. Almodóvar-Payá C, Guardiola-Ripoll M, Hostalet N, Giralt-López M, Arias B, Prats C, Gallego C, Millet S, Fañanás L, Pomarol-Clotet E, Fatjó-Vilas M. Common variants at plasticity gene NRN1 and its interaction with BDNF gene are associated with early-onset schizophrenia. Poster communication. *XX World Psychiatry Association (WPA) World Congress of Psychiatry.* May 10th-13th 2021, virtual. ++

30. Oscoz-Irurozqui M, **Almodóvar-Payá C**, Guardiola-Ripoll M, Guerrero-Pedraza A, Salgado-Pineda P, Sarró S, Pomarol-Clotet E, Fatjó-Vilas M. Cannabis use and cannabinoid receptor genes interaction modulates brain activity in first-episode psychosis. **Poster communication**. *XX World Psychiatry Association (WPA) World Congress of Psychiatry.* May 10th-13th 2021, virtual.

31. Guardiola-Ripoll M, **Almodóvar-Payá C**, Lubeiro A, Sotero A, Salgado-Pineda P, Salvador R, Papiol S, Ortiz-Gil J, Sarró S, McKenna PJ, Maristany T, Molina V, Pomarol-Clotet E, Fatjó-Vilas M. Epistatic effect of two schizophrenia genome-wide associated candidate genes (*CACNA1C* and *ZNF804A*) on working memory functional and behavioural response. **Oral communication**. *XX World Psychiatry Association (WPA) World Congress of Psychiatry.* May 10th-13th 2021, virtual.

32. Guardiola-Ripoll M, Sotero A, **Almodóvar-Payá C**, Hostalet N, Salgado-Pineda P, Salvador R, Ortiz-Gil J, Sarró S, McKenna PJ, Madre M, Pomarol-Clotet E, Fatjó-Vilas M. Effect of schizophrenia risk haplotypes of *DISC1* gene on working memory: a case-control neuroimaging association study response. **Oral communication**. *XX World Psychiatry Association (WPA) World Congress of Psychiatry.* May 10th-13th 2021, virtual.

2019

33. Fatjó-Vilas M, Guardiola-Ripoll M, **Almodóvar- Payá C**, Salgado-Pineda P, Lubeiro A, Alonso-Lana S, Ortiz-Gil J, Guerrero-Pedraza A, Sarro S, Pomarol-Clotet E. The role of KCNH2 gene on cognitive deficits in schizophrenia: a functional MRI study. **Poster communication**. *ResDem 2019: II International Conference on Cognitive Reserve in Dementia and other Disorders.* November 15th-16th 2019, Munich, Germany.

34. Oscoz-Irurozqui M, Guardiola-Ripoll M, **Almodóvar-Payá C**, Sarró S, Guerrero-Pedraza A, Pomarol-Clotet E, Fatjó-Vilas M. Cannabis use and genes of Dopaminergic and Endocannabinoid systems: their role in psychotic symptoms and cognition in First-episode Psychosis. **Poster**

communication. *XIX World Psychiatric Association (WPA) World Congress*. August 21st-24th 2019, Lisbon, Portugal.

35. Oscoz-Irurozqui M, **Almodóvar-Payá C**, Guardiola-Ripoll M, Guerrero-Pedraza A, Salgado-Pineda P, Sarró S, Pomarol-Clotet E, Fatjó-Vilas M. Cannabis use and CNR2 gene interaction modulates brain activity in first episode psychosis. **Poster communication**. *III World Congress and VI International Congress on Dual Disorders from the Spanish Society of Dual Pathology (SEPD)*. June 19th-22nd 2019, Madrid, Spain. ***Award to the best poster communication.**

36. Fuentes-Claramonte P, Santo-Angles A, Salvador R, Argila I, Albacete A, Guardiola-Ripoll M, **Almodóvar-Payá C**, Ramiro N, Boix E, Salgado-Pineda P, Bosque C, Torres ML, Panicalli F, Sarri C, Portillo F, McKenna PJ, Pomarol-Clotet E. Negative symptoms in schizophrenia as a deficit in goal management: an fMRI study. **Poster communication**. *2019 OHBM Annual Meeting of the Organization for Human Brain Mapping*. June 9th-13th 2019, Rome, Italy.

37. Santo-Angles A, Fuentes-Claramonte P, Argila I, Guardiola-Ripoll M, **Almodóvar-Payá C**, McKenna PJ, Pomarol-Clotet E, Radua J. Reward and Fictive Prediction Error signals in the Ventral Striatum. **Poster communication**. *2019 OHBM Annual Meeting of the Organization for Human Brain Mapping*. June 9th-13th 2019, Rome, Italy.

2018

38. **Almodóvar-Payá C**, Guardiola-Ripoll M, Salgado-Pineda P, Gallego C, Prats C, Arias B, Pomarol-Clotet E, McKenna PJ, Fatjó-Vilas M. Evidence of NRN1 Gene Effect on Schizophrenia Age at Onset and Brain Activity. **Poster communication**. *XXVI World Congress of Psychiatric Genetics*. October 11th-15th 2018, Glasgow, United Kingdom. ++

39. Guardiola-Ripoll M, **Almodóvar-Payá C**, Salgado-Pineda P, Lubeiro A, Alonso-Lana S, Ortiz-Gil J, Guerrer-Pedraza A, Sarró S, Pomarol-Clotet E Fatjó-Vilas M. Effect of KCNH2 and CACNA1C on Cognitive Performance and Brain Activity: Genetic Association Study in Schizophrenia Patients and Healthy Subjects. **Poster communication**. *XXVI World Congress of Psychiatric Genetics*. October 11th-15th 2018, Glasgow, United Kingdom.

40. **Almodóvar-Payá C**, Guardiola-Ripoll M, Salgado-Pineda P, Moreno M, Gallego C, Prats C, Arias B, Pomarol-Clotet E, McKenna PJ, Fatjó-Vilas M. NRN1 and functional MRI: association analysis in Schizophrenia Patients and Healthy Subjects. **Poster communication**. *6th Biennial*

Schizophrenia International Research Society Conference 2018. April 4th-8th 2018, Florence, Italy. ++

41. Guardiola-Ripoll M, **Almodóvar-Payá C**, Lubeiro A, Alonso-Lana S, Ortiz-Gil J, Guerrero-Pedraza A, Sarró S, Pomarol-Clotet E, Fatjó-Vilas M. Calcium and potassium voltage-gated channels genes association analysis: evidence on their role in cognitive performance of schizophrenia patients and healthy subjects. **Poster communication**. *6th Biennial Schizophrenia International Research Society Conference 2018*. April 4th-8th 2018, Florence, Italy.

National:

2024 1. Almodóvar-Payá C. *Identificación y búsqueda de potenciales biomarcadores para una nueva tipificación y abordaje terapéutico de las psicosis.* **Oral communication.** ***XXVII Congreso Nacional de Psiquiatría.*** October 17th-19th 2024, San Sebastián, Spain.

2020 2. Oscoz-Irurozqui M, Guardiola-Ripoll M, **Almodóvar-Payá C**, Sarró S, Guerrero-Pedraza A, Pomarol-Clotet E, Fatjó-Vilas M. Cannabis use and genes of the endocannabinoid system: their role in psychotic symptoms and cognition in first-episode psychosis (Oral communication). *XXII Congreso de Patología Dual*. November 16th-19th 2020, virtual. ****Award to the best poster communication.**

2017 3. Almodóvar-Payá C, Salgado-Pineda P, Gallego C, Arias B, Pomarol-Clotet E, McKenna PJ, Fatjó-Vilas M. *NRN1* gene and brain structure: an association study in patients with schizophrenia and healthy subjects. **Oral communication.** *XX Congreso de la Sociedad Española de Antropología Física*. July 12th-14th 2017, Barcelona, Spain. ****Award to the best oral communication.**

NATIONAL WORKSHOPS & SEMINARS COMMUNICATIONS

I have participated as **co-author of 13 scientific communications at national seminars and workshops**, 4 of which as the first presenting author (1 poster and 3 oral communications). Four of these communications are directly related to my doctoral thesis project (marked with ++). Moreover, I have received as the first presenting author an **award** for the best oral communication (marked with **).

2023 1. Guardiola-Ripoll M, **Almodóvar-Payá C**, Arias A, Latorre-Guardia M, Canales-Rodríguez EJ, García-León MA, Fuentes-Claramonte P, Salvador R, Pomarol-Clotet E, Papiol S, Fatjó-Vilas M. The evolutionary traces behind schizophrenia: Human Accelerated Regions

(HARs) linked to foetal neurodevelopment modulate cortical surface area in patients with the disorder. **Oral communication.** *X Laboratorio de Ideas CIBERSAM 2023*. April 20th-21st 2023, Reus, Spain.

2022

2. Sotero-Moreno A, Guardiola-Ripoll M, Moreira M, Giralt-López M, **Almodóvar-Payá C**, Hostalet N, Miret S, Campanera S, Muñoz MJ, Salvador R, Fañanás L, Pomarol-Clotet E, Fatjó-Vilas M. Disrupted in Schizophrenia 1 gene (DISC1) as a potential mediator of dermatoglyphic neurodevelopmental markers in schizophrenia. **Oral communication**. *XII Simposi de Neurobiologia: Cap a la Medicina Traslacional*. Societat Catalana de Biologia. May 13th 2022, Barcelona, Spain.

3. Latorre-Guardia M, **Almodóvar-Payá C**, Arias B, Penadés R, Guardiola-Ripoll M, García-León MA, Fuentes-Claramonte P, Pomarol-Clotet E, Fatjó-Vilas M. FKBP5 methylation patterns: cortical thickness and clinical correlates in schizophrenia. Oral communication. *XII Jornada de Cromatina i Epigenètica de la Societat Catalana de Biologia*. May 13th 2022, Barcelona, Spain.

4. Guardiola-Ripoll M, Sotero-Moreno A, Kebir O, Chaumette B, **Almodóvar-Payá C**, Hostalet N, Moreira M, Giralt M, Salvador R, Odile-Krebs M, Fatjó-Vilas M. Endocannabinoid system genetic variability role on dermatoglyphic patterns and schizophrenia-spectrum disorders. **Oral Communication**. *5th Biomed PhD Day*. February 10th 2022, Barcelona, Spain.

2021

5. **Almodóvar-Payá C,** Latorre-Guardia M, Arias B, Penadés R, Guardiola-Ripoll M, Fuentes-Claramonte P, García-León MA, Pomarol-Clotet E, Fatjó-Vilas M. *BDNF* and *COMT* methylation patterns and brain structural correlates in schizophrenia. **Poster communication.** *VIII Laboratorio de Ideas CIBERSAM 2021*. May 25th-27th 2021, virtual. ++

6. Guardiola-Ripoll M, Sotero A, **Almodóvar-Payá C**, Hostalet N, Salvador R, Pomarol-Clotet E, Fatjó-Vilas M. Novel findings on the pathophysiological mechanisms in schizophrenia: studies on the brain functional effects of haplotypic variability and genetic epistasis. **Oral communication**. *VIII Laboratorio de Ideas CIBERSAM 2021*. May 25th-27th 2021, virtual.

7. Latorre-Guardia M, **Almodóvar-Payá C**, Penadés R, AriasB, Guardiola-Ripoll M, Fuentes-Claramonte P, García-León MA, Pomarol-Clotet E, Fatjó-Vilas M. Impact of COMT and BDNF epigenetic profiles on brain cortex in schizophrenia. **Oral communication.** *XI Jornada de Cromatina*

i Epigenética de la Societat Catalana de Biologia. May 14th 2021, Barcelona, Spain.

2018

8. Almodóvar-Payá C, Guardiola-Ripoll M, Salgado-Pineda P, Gallego C, Prats C, Arias B, Pomarol-Clotet E, McKenna PJ, Fatjó-Vilas M. The role of Neuritin gene in modulating schizophrenia age at onset and brain activity during a working memory task. Oral communication. *XI Simposi de Neurobiologia de la Societat Catalana de Biologia*. November 12th-13th 2018, Barcelona, Spain. ++
9. Guardiola-Ripoll M, **Almodóvar-Payá C**, Salgado-Pineda P, Lubeiro A, Alonso-Lana S, Ortiz-Gil J, Guerrer-Pedraza A, Sarró S, Pomarol-Clotet E Fatjó-Vilas M. Genetic variability in neural excitability genes modulates cognitive performance and brain activity a case-control study in schizophrenia. **Oral communication**. *XI Simposi de Neurobiologia de la Societat Catalana de Biologia*. November 12th-13th 2018, Barcelona, Spain.
10. Oscoz-Irurozqui M, Guardiola-Ripoll M, **Almodóvar-Payá C**, Sarró S, Guerrero-Pedraza A, Pomarol-Clotet E Fatjó-Vilas M. Variabilidad en genes del sistema dopaminérgico y cannabinoide y su asociación con el consumo de cannabis en pacientes con un primer episodio psicótico: evidencias preliminaries. **Poster communication**. *Jornada Cloenda 2017-2018 de la Societat Catalana de Psiquiatria i Salut Mental*. June 1st 2018, Sitges, Spain.
11. Guardiola-Ripoll M, **Almodóvar-Payá C**, Salgado-Pineda P, Lubeiro A, Alonso-Lana S, Ortiz-Gil J, Guerrero-Pedraza A, Sarró S, Pomarol-Clotet E, Fatjó-Vilas M. *CACNA1C* and *KCNH2* effect on Cognitive Performance and Brain Activity in Schizophrenia patients and healthy subjects: Preliminary results. **Oral communication**. *VI Laboratorio de Ideas CIBERSAM 2018*. May 31st - June 1st 2018, San Fernando, Spain.

2017

12. Almodóvar-Payá C, Salgado-Pineda P, Callado LF, Gallego C, Arias B, Pomarol-Clotet E, McKenna PJ, Fatjó-Vilas M. Analysis of neuroimaging and brain expression correlates of NRN1 gene in schizophrenia. Oral communication. *V Edición Laboratorio de Ideas CIBERSAM 2017*. June 1st-2nd 2017, Santander, Spain. **Award to the best oral communication. ++

SPECIALIZED TRAINING

I have attended **23 specialized courses**, most of them related with neuroscience, psychiatry, genetics, neuroimaging, and statistics.

2024
1. XXI Curso Intensivo CIBERSAM de Introducción a la Investigación en Neurociencias. Entity: Consorcio CIBER para el área temática de Salud Mental (CIBERSAM). September 20th 2024, Barcelona, Spain.

2023
2. XX Curso Intensivo CIBERSAM: "Redefiniendo la enfermedad mental: La Esquizofrenia" (6h). Entity: Consorcio CIBER para el área temática de Salud Mental (CIBERSAM). September 9th 2023, Barcelona, Spain.
3. WORKSHOP: Data-Science para biociencias (R, Python, Bioinformática) (9h). Entity: Institut de Recerca de la Biodiversitat (IRBio), grup de recerca en Estadística, Ciència de les Dades i Bioinformàtica, Departament de Genètica, Microbiologia i Estadística, Facultat de biología, Universitat de Barcelona (UB). July 5th 2023, Barcelona, Spain.
4. XI FORO CIBERSAM DE INVESTIGACIÓN EN PSIQUIATRÍA (11h). Entity: Consorcio CIBER para el área temática de Salud Mental (CIBERSAM). June 8th 2023, Barcelona, Spain.

2022
5. XIX curso intensivo de introducción a la investigación en neurociencias: The prenatal, perinatal and infant environmental risk factors in mental disorders (7h). Entity: Consorcio CIBER para el área temática de Salud Mental (CIBERSAM). September 9th 2022, Barcelona, Spain.
6. Curso REDCap (20h). Entity: x7 Perseventh. January 10th-21th 2022, Online.

2021
7. X Foro Internacional Cibersam de Investigación en Psiquiatría (11h). Entity: Consorcio CIBER para el área temática de Salud Mental (CIBERSAM). November 23th-25th 2021, Online.
8. Jornada científica: Utilización de muestras cerebrales humanas en el estudio de enfermedades psiquiátricas (5h). Entity: Consorcio CIBER para el área temática de Salud Mental (CIBERSAM). November 11th 2021, Online.
9. XXIV Congreso Nacional de Psiquiatria (13h). Entitiy: Sociedad Española de Psiquiatria. October 28th-30th 2021, Valencia, Spain.
10. XVIII curso intensivo de introducción a la investigación en neurociencias: actualización en la evaluación, intervención e investigación del maltrato infantil al principio de la vida (9h). Entity: Consorcio CIBER para el área temática de Salud Mental (CIBERSAM). June 28th-29th 2021, Barcelona, Spain.

11. Jornada Científica: Descodificant el DNA, avenços i nous reptes per al futur (2,5h). Entity: Societat Catalana de Biología. January 11th 2021, Online.

2020

12. IX foro internacional CIBERSAM de investigación en psiquiatría (11h). Entity: Consorcio CIBER para el área temática de Salud Mental (CIBERSAM). November 17th-19th 2020, Online.

2019

13. Introduction to GWAS (Genome-Wide Association Studies (36h). Entity: Transmitting Science. November 25th-29th 2019, Barcelona, Spain.
14. III Curso de Actualización en Psicosis de inicio en la Infancia y la Adolescencia (6h). Entity: Servicio de Psiquiatría y Psicología (Hospital Sant Joan de Déu) y Consorcio CIBER para el área temática de Salud Mental (CIBERSAM). May 17th 2019, Barcelona, Spain.
15. XVII Curso Intensivo de Introducción a la Investigación en Neurociencias: "Neuroendocrinologia del Trastorno Mental Infanto-Juvenil (6h). Entity: Consorcio CIBER para el área temática de Salud Mental (CIBERSAM). May 10th 2019, Barcelona, Spain.

2018

16. V Curso de Técnicas de Neuroimagen Avanzada (80h). Entity: FIDMAG Germanes Hospitalàries Research Foundation. November 2nd 2018 – April 12th 2019, Sant Boi de Llobregat, Spain.
17. V Curso de Estadística Básica para Ciencias de la Salud con Excel (16h). Entity: FIDMAG Germanes Hospitalaries Research Foundation. May 26th – Setember 2nd 2018, Barcelona, Spain.
18. XVI Curso Intensivo de Introducción a la Investigación en Neurociencias: Estrés Oxidativo e Inflamación en Enfermedad Mental: ¿Causa o Consecuencia? (7h). Entity: Consorcio CIBER para el área temática de Salud Mental (CIBERSAM). April 27th 2018, Barcelona, Spain.
19. VIII Jornadas Unidad de Crisis de Adolescentes: Maltrato en la Infancia y Adolescencia (6,5h). Entity: FIDMAG Germanes Hospitalaries Research Foundation. February 28th 2018, Sant Boi de Llobregat, Spain.

2017

20. B·DEBATE. Natural Selection in Humans: Understanding our adaptations (15h). Entity: Biocat, "la Caixa" Foundation y Insititute of Evolutionary Biology (IBE). July 17th-18th 2017, Barcelona, Spain.
21. XV Curso Intensivo de Introducción a la Investigación en Neurociencias: The Early Origin of Mental Health. Hours: 6h. Responsable: L. Fañanás. Awarding entity: Consorcio CIBER para el

área temática de Salud Mental (CIBERSAM). June 28th 2017, Barcelona, Spain.

22. II Curso de Actualización en Psicosis de inicio en la Infancia y la Adolescencia (8h). Entity: Consorcio CIBER para el área temática de Salud Mental (CIBERSAM). April 21th 2017, Barcelona, Spain.
23. XIV Curso Intensivo de Introducción a la Investigación en Neurociencias: Cannabis y Enfermedad Mental (8h). Entity: Consorcio CIBER para el área temática de Salud Mental (CIBERSAM). January 27th 2017, Barcelona, Spain.

TEACHING EXPERIENCE

I have taught a total of **249h (in bachelor's and master's degrees)**, I have **co-directed 4 master's theses and 2 research stays**. Moreover, I have been part of the panel of bachelor's (6 students) and master's degree final projects (4 students).

Additionally, I also coordinate the rotation of the internal resident nurses from Benito Menni Hospital through the FIDMAG research unit.

Supervision of final projects:

2023 – 2024 1. **Master's thesis co-direction.** Master's degree in Biological Anthropology. University of Barcelona (UB) and Autonomous University of Barcelona (UAB). Title: *Neuroimaging-genetic study on the interplay between two schizophrenia-related genes: NRN1 and its novel described receptor NPTN*. Student: Yejin Mori.

2023 – 2022 2. **Master's thesis co-direction.** Master's degree in Introduction to Research in Mental Health. University of Barcelona (UB), Autonomous University of Barcelona (UAB), Universidad Complutense de Madrid (UCM), Universidad de Cádiz (UCA), Centro de Investigación Biomédica en Red en Salud Mental (CIBERSAM). Title: *Cognitive remediation in schizophrenia: effects on patterns of methylation in the NRN1 gene*. Student: Irene Gómez Alonso. **Qualification: Graded with Honors**. July 14th 2023.

2021 – 2020 3. **Master's thesis co-direction.** Master's Degree in Neuroscience. University of Barcelona (UB). Title: *The role of BDNF and COMT genes in schizophrenia: analysis of genetic and methylation variability and their neuroanatomical correlates*. Student: Mariona Latorre Guardia. **Qualification: Graded with Honors**. September 7th 2021.

2020 – 2019 4. **Master's thesis co-direction.** Master's degree in Biological Anthropology. University of Barcelona (UB) and Autonomous

University of Barcelona (UAB). Title: *The role of NRN1 gene in schizophrenia: analysis of its genetic variability in cortical thickness.* Student: Enric Garcia Torrents. **Qualification: Graded with Honors.** September 14th 2020.

Bachelor's & master's degree teaching activities:

2024 - 2025 *First semester*

1. **Master's degree in human anthropology**. Course: **Laboratory II**. Course format: **Lectures**. Course type: Elective (1st semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 10h**.
2. **Bachelor's degree in biomedical science.** Course: **Biology: Introduction to Biomedicine**. Course format: **Tutoring and problem-solving session**. Course type: Required (1st semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 12h.**

Total hours academic course 2023 - 2024: 22h

2023 - 2024 *First semester*

3. **Bachelor's degree in biomedical science.** Course: **Genetic Basis of Diseases**. Course format: **Tutoring and problem-solving session**. Course type: Required (1st semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 4h.**
4. **Bachelor's degree in biomedical science.** Course: **Biology: Introduction to Biomedicine**. Course format: **Tutoring and problem-solving session**. Course type: Required (1st semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 12h.**
5. **Bachelor's degree in biomedical science.** Course: **Genetic Basis of Diseases**. Course format: **Laboratory practice**. Course type: Required (1st semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 10h.**
6. **Master's degree in human anthropology**. Course: **Basic Knowledge in Biological Anthropology: Key Concepts**. Course format: **Lectures**. Course type: Elective (1st semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 5h**.
7. **Master's degree in human anthropology**. Course: **Laboratory II**. Course format: **Lectures**. Course type: Elective (1st semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 10h**.

8. **Master's degree in human anthropology**. Course: **Laboratory II**. Course format: **Laboratory practice**. Course type: Elective (1st semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 20h**.

Second semester

9. **Bachelor's degree in biology.** Course: **Biological Anthropology**. Course format: **Laboratory practice**. Course type: Required (2nd semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 33h.**
10. **Master's degree in human anthropology**. Course: **Molecular Techniques Applied to Anthropology**. Course format: **Laboratory practice**. Course type: Elective (2nd semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 20h**.
11. **Master's degree in human anthropology**. Course: **Genetic Epidemiology I**. Course format: **Laboratory practice**. Course type: Elective (2nd semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 20h**.
12. **Master's degree in human anthropology**. Course: **Genetic Epidemiology II**. Course format: **Laboratory practice**. Course type: Elective (2nd semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 20h**.

Total hours academic course 2023 - 2024: 94h

2022 – 2023 *Second semester*

13. **Bachelor's degree in biology.** Course: **Biological Anthropology**. Course format: **Laboratory practice**. Course type: Required (2nd semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 44h.**
14. **Master's degree in human anthropology**. Course: **Genetic Epidemiology I**. Course format: **Lectures**. Course type: Elective (2nd semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 10h**.
15. **Master's degree in human anthropology**. Course: **Genetic Epidemiology II**. Course format: **Lectures**. Course type: Elective (2nd semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 14h.**
16. **Master's degree in human anthropology**. Course: **Molecular Techniques Applied to Anthropology**. Course format: **Lectures**.

Course type: Elective (2nd semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 17h**.

Total hours academic course 2022 - 2023: 85h

Panel member of the bachelor's degree final projects: 6 students

Panel member of the master's degree final projects: 4 students

2021 - 2022 *Second semester*

17. **Master's degree in human anthropology**. Course: **Genetic Epidemiology II**. Course format: **Lectures**. Course type: Elective (2nd semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 2h.**

Total hours academic course 2021 - 2022: 2h

2020 – 2021 *Second semester*

18. **Bachelor's degree in biology.** Course: **Biological Anthropology**. Course format: **Lectures**. Course type: Required (2nd semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 20h.**
19. **Master's degree in human anthropology**. Course: **Genetic Epidemiology I**. Course format: **Lectures**. Course type: Elective (2nd semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 7h.**
20. **Master's degree in human anthropology**. Course: **Genetic Epidemiology II**. Course format: **Lectures**. Course type: Elective (2nd semester). Location: Faculty of Biology, University of Barcelona (UB). **Total hours: 9h**.

Total hours academic course 2020 - 2021: 36h

Experience supervising research stays:

2021
1. **Stay supervision.** Master's degree in Introduction to Research in Mental Health. University of Barcelona (UB), Autonomous University of Barcelona (UAB), Universidad Complutense de Madrid (UCM), Universidad de Cádiz (UCA), Centro de Investigación Biomédica en Red en Salud Mental (CIBERSAM). Student: Blanca Ruiz de La Torre Reyes. March 29th – April 2nd 2021.

2020
2. **Stay supervision.** Master's degree in Introduction to Research in Mental Health. University of Barcelona (UB), Autonomous

University of Barcelona (UAB), Universidad Complutense de Madrid (UCM), Universidad de Cádiz (UCA), Centro de Investigación Biomédica en Red en Salud Mental (CIBERSAM). Student: Susana Lira Rueda. April 20th-24th 2020.

Tutoring Internal Nursing Resident rotation from Benito Menni CASM at FIDMAG

2023 – 2024
1. Student: Eva Heredero Salán. Dates: January 16th – April 2nd 2024
2. Student: Marco Hernández Muriel. Dates: January 16th – April 2nd 2024
3. Student: María Vaz Tejido. Dates: September 6th – 6th December 2023

2022 – 2023
4. Student: Raquel Valdovinos Garrido. Dates: May 3thr – July 26th 2023

High school teaching activities:

- **Almodóvar-Payá C** and Guardiola M. Cannabis, genética y trastornos mentales. ¿Tienen algo que ver?. Conference integrated in the Aprenentatge i Serveis (ApS) Educative Programme. IES Francisco Goya. May 15th 2018, Barcelona, Spain.
- **Almodóvar-Payá C** and Esperanza E. Com som, qui som, què serem: està tot escrit als nostres gens?. Conference integrated in the Aprenentatge i Serveis (ApS) Educative Programme. IES l'Alzina, May 5th 2016, Barcelona, Spain.

ORGANIZATION OF RESEARCH & DEVELOPMENT ACTIVITIES

- Trobada entre científiques de la UB i noies estudiants d'instituts.
- Member of the Organizer Committee of the IV Biomed PhD Day. Congress organized by students of the Biomedicine Doctoral Program. January 14th 2020, Barcelona, Spain.