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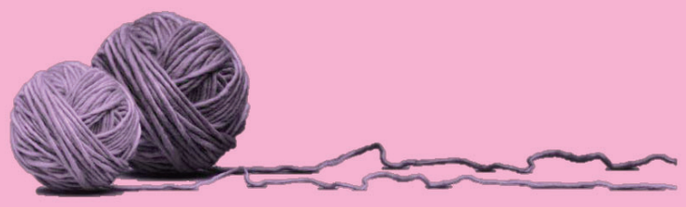
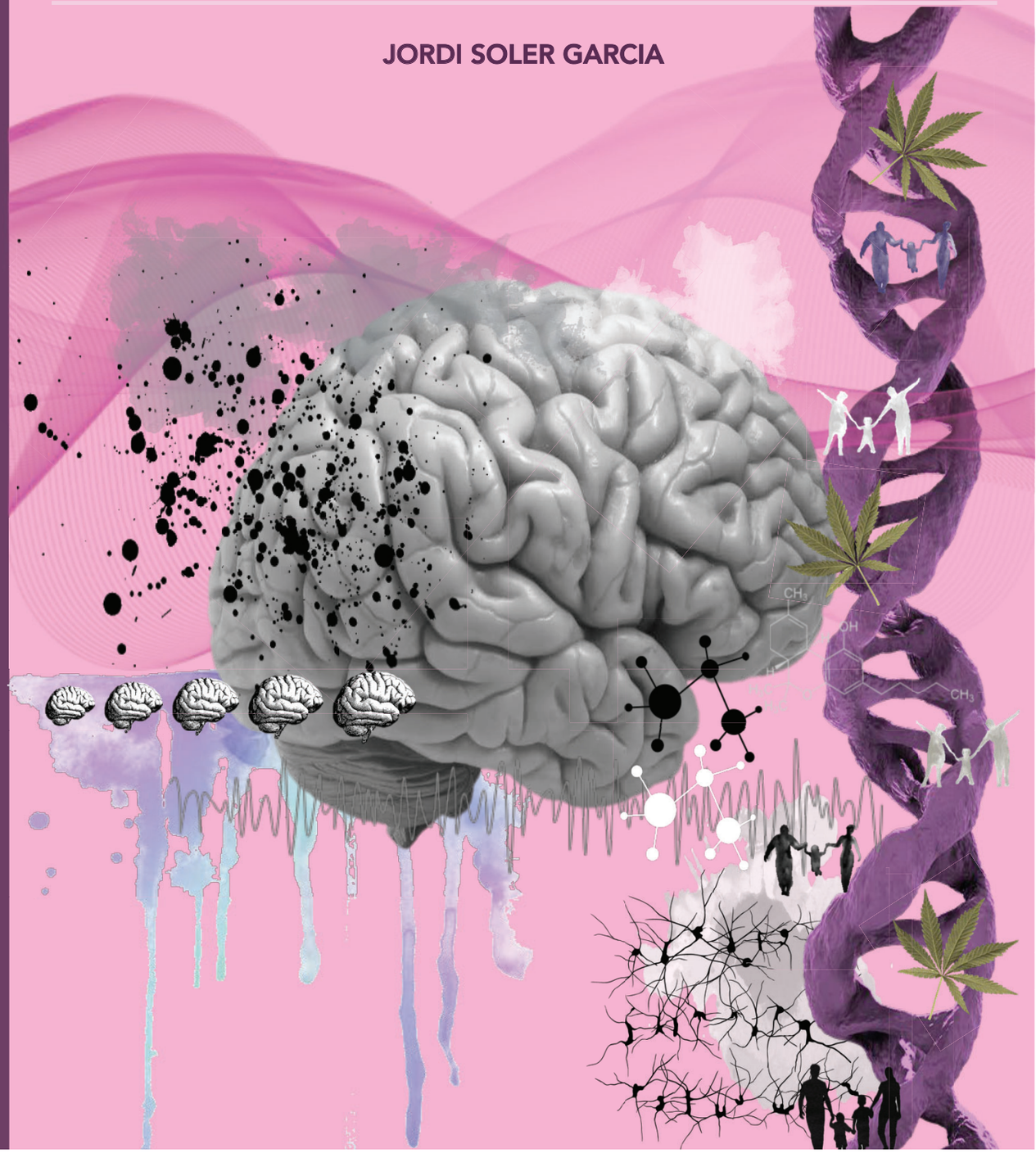


DOCTORAL THESIS ★ JORDI SOLER GARCIA ★ 2021

TOWARDS DISENTANGLING THE CLINICAL HETEROGENEITY AND GENETIC COMPLEXITY OF PSYCHOTIC DISORDERS:

FROM FAMILY-BASED APPROACHES TO GENE-ENVIRONMENT STUDIES

JORDI SOLER GARCIA



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Towards disentangling the genetic complexity and clinical heterogeneity of psychotic disorders:

from family-based approaches to gene-environment studies

Doctoral Thesis presented by

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As a requirement to apply for the degree of PhD
by the **Universitat de Barcelona**

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ABSTRACT

Psychotic disorders are psychiatric conditions with a worldwide prevalence of around 4% and a tremendous personal, economic and social burden. As complex phenotypes, psychotic disorders are caused by multiple genetic variants, environmental factors and their interaction.

According to this, a better understanding of the genetic and environmental influences underlying these disorders may provide a way to dissect the biology of psychosis and, ultimately, allow developing novel therapies. However, the study of the aetiological basis of schizophrenia and other psychotic disorders, though, has a serious limitation in the high biological heterogeneity underlying these pathologies. The heterogeneity of clinical profiles and the high phenotypic variability, in turn, causes uncertainty on the genetic results related to these disorders. Thus, the reduction of phenotypic complexity has become an essential step to contribute to the genetic dissection of brain complex phenotypes.

The present dissertation aimed to contribute disentangling the heterogeneity of psychotic disorders by means of different approaches: the use of family-based studies, the use of psychosis-associated intermediate phenotypes and the use of gene-environment interaction studies. Three specific hypotheses related to these approaches have been tested, giving rise to six manuscripts submitted to international peer reviewed journals.

The results of the present thesis reveal that the combined use of family-based designs and intermediate phenotypes related to psychosis may facilitate the identification of more homogeneous forms of psychotic disorders in terms of genetic aetiology. Thus, by means of this strategy, two different subclinical phenotypes such as schizotypy (a set of personality traits) and the cognitive dimension of attention and working memory have been identified as familial vulnerability markers for psychosis in samples of families affected with schizophrenia and bipolar disorder, respectively. The study of the familial aggregation pattern of these phenotypes have lead to the identification of subgroups of families with similar phenotypic –and, therefore also genotypic– profiles.

Moreover, by using family-based association designs, different genes involved in the modulation of synaptic plasticity (*DAOA*, *ZNF804A, AKT1*) have been associated with the risk for psychosis, as measured with the expression of intermediate phenotypes, including schizotypy and cognitive performance.

Also, results from this thesis provide evidence of the role genetic variability on cognitive performance and also as a modulator of the effect of cannabis use on the variance of other intermediate phenotypes. Particularly, it has been revealed the effect of *AKT1* gene on attentional processes and, also, the effect of *ZNF804A* gene on the expression of schizotypy conditional to the cannabis use.

Despite the last advances in the comprehension of the aetiology of psychosis, the identification of the involved genetic factors has still a long way to go. Thus, it is necessary to continue making efforts towards understanding the aetiopathogenic basis of psychotic disorders, taking into account both genetic and environmental factors. The present dissertation has intended to provide our grain of sand to the collective construction of knowledge on the aetiology of psychosis by means of using different strategies that have proven to contribute to elucidating the heterogeneity underlying these disorders, which in turn might lead to an improvement of the identification of the underlying causal genetic variants.

RESUM

Els trastorns psicòtics són trastorns mentals amb una prevalença mundial del 4% i amb una gran càrrega personal, econòmica i social associada, que estan causats per factors genètics, factors ambientals i la interacció d'ambdós durant diferents etapes del desenvolupament cerebral.

Si volem disposar de noves teràpies que ajudin a millorar la qualitat de vida de les persones amb algun diagnòstic psicòtic, és evident que abans ens cal entendre millor les contribucions que la càrrega genètica i la càrrega ambiental de cada individu tenen en el desenvolupament d'aquests trastorns. Malauradament, l'estudi de les bases etiològiques de l'esquizofrènia i d'altres trastorns psicòtics està condicionada per la gran heterogeneïtat clínica que presenten aquests fenotips. Per tant, per tal de seguir avançant en la identificació dels factors genètics associats al risc per a psicosi, és necessari conèixer i abordar els factors relacionats amb aquesta heterogeneïtat.

En aquest sentit, aquesta tesi pretén contribuir a abordar aquesta heterogeneïtat intrínseca als trastorns psicòtics a través de diferents estratègies: l'ús de mostres de famílies, l'ús de fenotips intermedis associats al risc per a psicosi i l'estudi de la interacció gen-ambient en l'etiologia d'aquests trastorns. En aquesta tesi s'han plantejat tres hipòtesis diferents, que han donat lloc a 6 articles publicats en revistes científiques internacionals.

Els resultats inclosos en la tesi revelen que l'anàlisi de fenotips intermedis associats al risc per psicosi en mostres de famílies facilita la identificació de formes més homogènies de psicosi, en relació a la seva etiologia genètica. Així doncs, mitjançant aquesta estratègia s'han identificat dos fenotips subclínics, com són la esquizotípia (trets de personalitat) i la dimensió cognitiva de l'atenció i la memòria de treball, com a marcadors de vulnerabilitat familiar per a psicosi en una mostra de famílies afectades amb esquizofrènia i en una de famílies afectades amb trastorn bipolar, respectivament. L'estudi del patró d'agregació familiar d'aquests fenotips ha permès la identificació de subgrups de famílies amb perfils fenotípics –i, per tant, també genotípics– similars.

A més, en mostres clíniques i també no-clíniques, s'ha identificat la relació d'alguns gens relacionats amb la plasticitat sinàptica (*DAOA, ZNF804A, AKT1*) amb el risc de patir psicosi, mesurat a través de l'expressió de diferents fenotips intermedis com els nivells d'esquizotípia o el rendiment cognitiu.

Els resultats inclosos en aquesta tesi també evidencien el paper que la variabilitat genètica té sobre el rendiment cognitiu i, alhora, com a modulador de l'efecte que el consum de cannabis té sobre l'expressió de diferents fenotips intermedis. En particular, s'ha descrit l'efecte del gent *AKT1* en l'atenció i també l'efecte del gen *ZNF804A* sobre l'expressió de l'esquizotípia condicionat al consum de cannabis.

Tot i els últims avenços en la comprensió de la etiologia de la psicosi, la investigació dels factors genètics causants de la psicosi i la seva interacció amb els factors ambientals encara té un llarg camí per recórrer. Aquesta tesi ha pretès aportar un granet de sorra a la construcció col·lectiva d'aquest coneixement mitjançant l'ús de diferents estratègies, les quals han demostrat que poden ser molt útils per tal d'abordar d'una manera més eficaç l'heterogeneïtat clínica inherent als trastorns psicòtics i poder millorar la identificació de les variants genètiques i ambientals implicades.

GLOSSARY

Some words throughout the text are marked with the symbol ⓘ. Their definitions are given below in alphabetical order.

- **Aetiology:** The causes of a disorder.
- **Biomarker:** A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to an intervention.
- **Complex disorder:** Disorder caused by genetic factors, environmental factors and their interaction.
- **Copy number variant (CNV):** gain or loss of genomic material of at least 1 kilobase in size that can encompass a single exon of a gene, an entire gene or even multiple genes.
- **Disability-adjusted life years (DALYs):** DALY is the summary measure used to indicate the overall burden of disease due to the number of years lost due to poor health, disability or premature mortality. One DALY represents the loss of the equivalent of one year of full health.
- **Diagnosis:** The act of identifying a disease from its signs and symptoms.
- **Etiopathogeny:** The cause and development of a disease or abnormal condition.
- **Familiality:** Also known as familial aggregation, is the clustering of certain traits, behaviours, or disorders within a given family. Family aggregation may arise because of genetic or environmental similarities.
- **Genetic architecture:** the overall composition of the implicated genetic risk variants in the population; this is, the total number of variants associated with the disorder, the degree of risk conferred by these variants and the frequencies in affected individuals and the general population.
- **Genotype:** The genetic makeup of an organism. In other words, it describes an organism's complete set of genes. In a more narrow sense, the term can also be used to refer to the particular combination of alleles for a particular gene or locus.

- **Genome-wide association studies (GWAS):** Hypothesis-free approaches where millions of variants are compared between cases and control subjects to explore whether particular genetic variants are found more frequently in patients than in controls.
- **Heritability:** Estimate that indicates the proportion of phenotypic variability that is attributable to genetic factors: higher estimates suggest that genetic variability has a large influence on the variability of a given trait in the population.
- **Missing heritability:** Phenomenon in which the heritability estimated by single genetic variants (SNPs) do not account for much of the heritability of a disorder or phenotype that is estimated from familiar and twin data.
- **Morbid risk:** Epidemiological estimate that indicates the probability of an individual born in a particular population to develop a disorder if she/he survives through the entire period of the risk for the disorder.
- **Odds ratio (OR):** Statistic that measures the association between an exposure and an outcome. The OR represents the odds with which an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure. Odds ratios are most commonly used in case-control studies.
- **Pathogenesis:** Biological mechanism/s that lead to a disorder.
- **Phenotype:** The set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.
- **Polygenic Risk Score:** Continuous score that use the sum of all known common variants to quantify the aggregate effect of common variants for a given disorder (e.g. schizophrenia) or trait (e.g. schizotypy, IQ, educational attainment). PRS is calculated by multiplying the number of risk alleles a person carries by the effect size of each allele and then, summing each of these products across all risk loci.
- **Population stratification:** It refers to differences in allele frequencies between cases and controls due to systematic differences in ancestry rather than the association of genes with the disease. Population stratification can result in false positives or negatives associations in genetic studies.

- **Prevalence:** Epidemiological index that measures the number of existing cases of a disease per 1000 persons at risk in a defined population at a specified time (e.g. point prevalence) or period (e.g. annual prevalence or lifetime prevalence).
- **Psychotic Disorder:** Disorders characterised by psychotic symptoms that cause a loss of contact with reality.
- **Single nucleotide polymorphism (SNP):** A single nucleotide polymorphism, or SNP (pronounced "snip"), is a variation at a single position in a DNA sequence among individuals that is present in at least 1% of the population.
- **Single nucleotide variant (SNV):** Variation at a single position in a DNA sequence among individuals present in less than 1% of the population.

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INTRODUCTION

1. Complexity and heterogeneity of psychotic disorders

1.1. Psychotic disorders

Psychiatric disorders are health conditions involving changes in emotion, thinking and/or behaviour that affect more than 21 million people worldwide. Psychiatric disorders account for a tremendous personal, educational, economic and societal burden, with 218 disability-adjusted life years (DALYs[①]) per 100.000, making this disorder the fifth leading cause of DALYs in the age group of 15-44 years (Murray et al., 2012; see **Figure 1**).

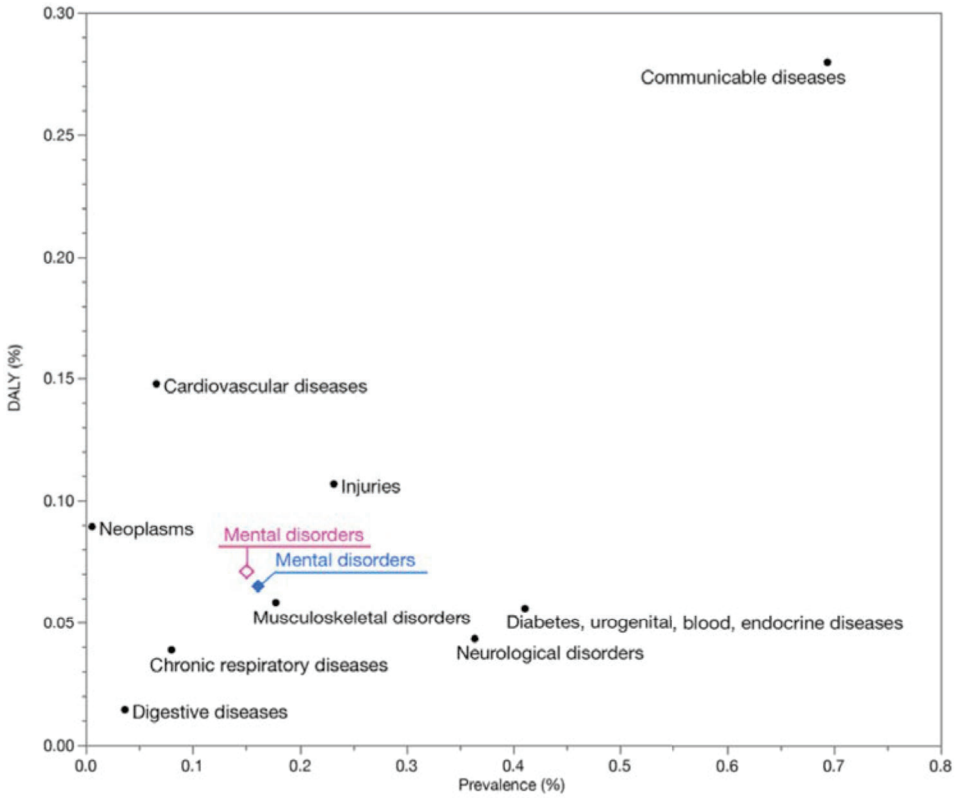


Figure 1. Prevalence and impact of psychiatric disorders compared to other major diseases. Prevalence (x axis) and disability-adjusted life years (DALYs, y axis) for 10 major classes of disorders are represented. Looking at both measures allows evaluation of how common and how impactful a psychiatric disorder is. Psychiatric disorders rank fifth and account for almost 7% (females are the open diamond and males are the closed diamond). Adapted from Sullivan and Geschwind (2019)**.**

Among psychiatric disorders, the present thesis turns the spotlight on psychotic disorders[①], in which distortions in thinking, perception, emotions, language and behaviour are core clinical features (American Psychiatric Association, 2013;

Arciniegas, 2015). The spectrum of psychotic disorders includes several diagnoses with a worldwide prevalence of around 4% (Perälä *et al.*, 2007; Bogren *et al.*, 2009). The Diagnostic and Statistical Manual of Mental Disorders (DSM) is one of the most used manual for the diagnosis of mental disorders. Given that schizophrenia is the most common psychotic disorder, they are also collectively termed as schizophrenia-spectrum disorders. However, many other diagnoses such as bipolar disorder are associated with psychostic symptoms (see **Table 1)**.

Table 1. Main DSM-5 diagnoses that course with psychotic symptoms

Diagnoses	Associated features
Delusional disorder	Isolated delusions in the absence of other psychotic symptoms
Brief psychotic disorder	Transient psychosis with return to premorbid function
Schizophreniform disorder	Sub-syndromal schizophrenia with multiple psychotic symptoms of duration more than 1 month and less than 6 months
Schizophrenia	Two or more psychotic symptoms for more than 6 months
Schizoaffective disorder	Psychotic symptoms for two weeks in the absence of mood symptoms and symptoms that meet criteria for a mood episode during a majority of the duration of illness
Substance/medication-induced psychotic disorder	Psychotic symptoms the direct result of a substance or medication
Psychotic disorder due to another medical condition	Psychotic symptoms the direct result of a medical condition
Catatonia	Used to describe psychiatric disorders but can have catatonia due to medical conditions
Other specified schizophrenia spectrum and other psychotic disorder	Other psychotic disorders that do not meet criteria for another disorder
Unspecified schizophrenia spectrum and psychotic disorder	Psychotic disorder due to unknown or undetermined causes
Bipolar disorder	Group of disorders (Bipolar I disorder, bipolar II disorder and cyclothymic disorder) that cause extreme fluctuation in a person's mood, energy, and ability to function and can course with psychosis

According to epidemiological data, the median incidence[Φ] of schizophrenia is 15.2/100.000 persons with a median rate ratio for males:females of 1.4:1; the lifetime prevalence[Φ] is 7.49 per 1.000 (Moreno-Küstner, Martín and Pastor, 2018) and the lifetime morbid risk[Φ] is 7.2 per 1000 (McGrath et al., 2008).

Apart from a significant disability, schizophrenia is associated with premature mortality, mainly due to suicide (lifetime prevalence of suicide attempts, 34.5%) (Suokas *et al.*, 2010; Walker, McGee and Druss, 2015; Hjorthøj *et al.*, 2017) and decreased fecundity (Power et al., 2013). Moreover, patients with a diagnosis of schizophrenia have a high prevalence of substance abuse (lifetime prevalence, 74%) (Lambert et al., 2005), homelessness (annual prevalence, 5%) (Folsom et al., 2005) and victimisation by others (prevalence over a 3-year period, 38%) (Brekke et al., 2001). A recent study has also revealed that subjects with schizophrenia have 2.7 times the odds of dying from coronavirus disease 2019 (COVID-19) after adjustment for known risk factors (Nemani *et al.*, 2021).

1.2. Clinical presentation of psychotic disorders

The onset of psychotic disorders characteristically occurs in adolescence or early adulthood, with males showing an earlier age at onset (Kessler *et al.*, 2007). It is usually frequently preceded by a prodromal phase, or high-risk state, characterised by impaired functioning and non-specific (e.g. attention problems, lack of energy, anxiety, anhedonia) and specific but attenuated symptoms of psychotic states (strange obsessions, abnormal perceptions, limited psychotic symptoms), in which the individual deviates from her/his stable premorbid level of functioning (Norman *et al.*, 2005; see **Figure 2**). To note, poor premorbid functioning, which may result from an altered brain development, is associated with the expression of psychosis and with a higher severity of psychotic symptoms (Lyngberg *et al.*, 2015; see section 1.4).

With regard to the clinical presentation, psychotic disorders include a range of symptoms that can be classified into three dimensions: positive symptoms, negative symptoms and cognitive impairments. Positive symptoms include hallucinations

(false perceptions), delusions (false beliefs), thought disorder and disorganised speech and behaviour. Negative symptoms include abolition (a lack of interest or engagement in goal-directed behaviour), social withdrawal, affective flattening and anhedonia (inability to experience pleasure). Finally, cognitive impairments include alterations in different cognitive domains, such as learning, attention, working memory or executive function (American Psychiatric Association, 2013).

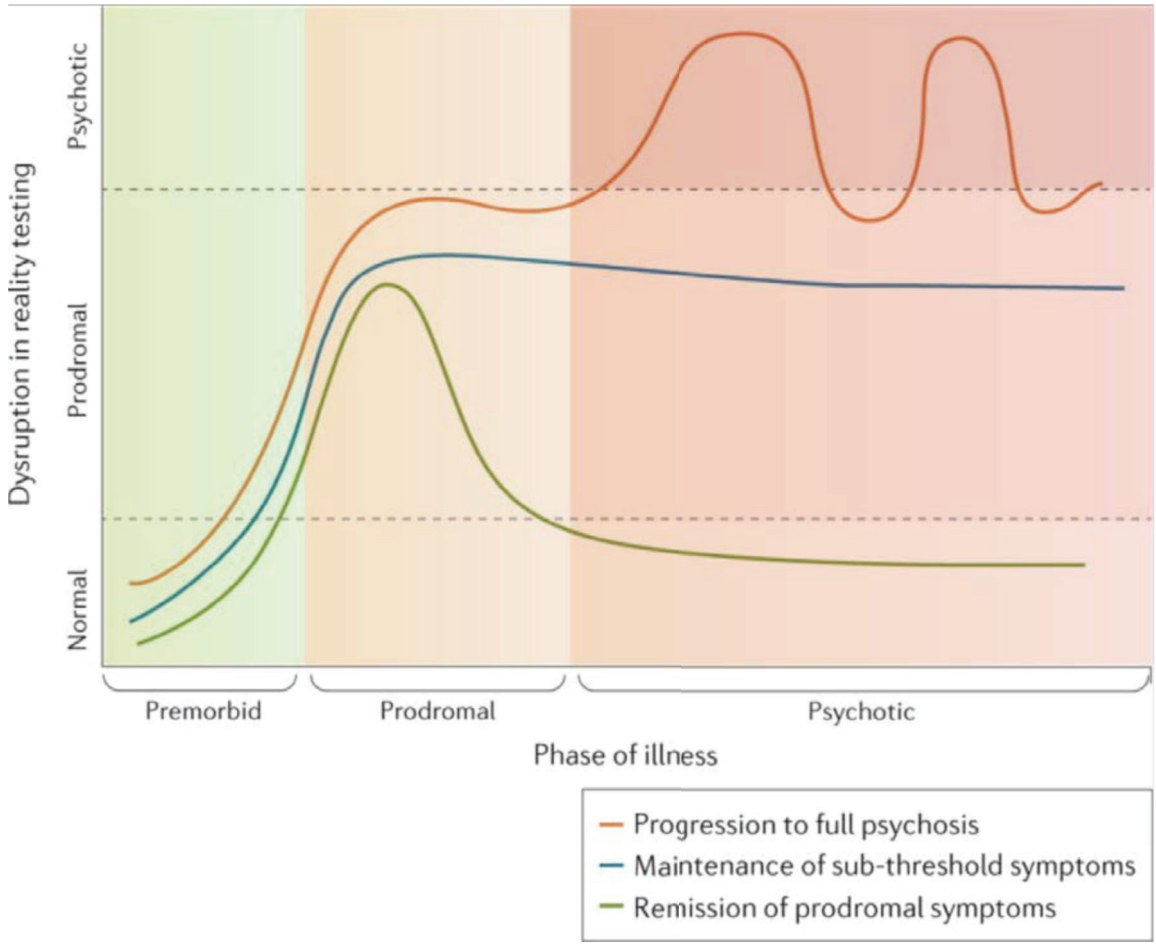


Figure 2. A descriptive model of the onset and course of psychotic symptoms among individuals who develop a prodromal risk syndrome. Approximately one-third of prodromal patients progress to full psychosis (orange line), one-third maintains stable levels of sub-threshold symptoms (blue line), and one-third remits the prodromal symptoms (green line). Adapted from Cannon (2015).

Psychotic disorders are characterised by heterogeneous phenotypes and variable courses, creating diverse symptom profiles to the point that the same diagnosis (e.g. schizophrenia) may identify individuals who share few or no symptoms (Van Rheenen *et al.*, 2017; Helldin *et al.*, 2020). In addition, patients with the same diagnosis differ widely in variables related to the longitudinal course of their illness (age at onset,

expression of signs and symptoms, etc.), leading to overall highly clinically heterogeneous disorders. As explained in section 1.5, the genetic component has a significant role in the development of psychotic disorders, and it is thought that this phenotypic heterogeneity might be a reflection of the genetic complexity underlying these pathologies.

The diagnosis of schizophrenia or other psychotic disorder requires confirmation that patients meet established criteria for the disorder and rule out other psychotic disorders or psychotic states. Once the diagnosis is made, the treatment entails multi-modal approaches, including medication, psychosocial interventions, and assistance with housing and financial sustenance (Tandon, Nasrallah and Keshavan, 2010). However, the clinical and genetic heterogeneity underlying each disorder also manifests in the patient's response to medications, frequently resulting in multiple changes in treatment strategy during the illness course (Lally and MacCabe, 2015). In this regard, a good understanding of the clinical heterogeneity and the etiopathogenic of the disorder is needed to achieve an individualised medicine, as it can be learnt from the path followed by other medicine fields (Gambardella *et al.*, 2020).

1.3. The psychosis continuum

The clinical continuity observed among schizophrenia-spectrum disorders has led to the notion of these disorders as different phenotypic manifestations of the same underlying aetiological processes, called the psychosis continuum (Keshavan *et al.*, 2011; DeRosse and Karlsgodt, 2015; see **Figure 3**). In this regard, both epidemiological and molecular approaches have reported an important overlap between diagnosis in terms of genetic liability, suggesting that these disorders share part of their underlying genetic architecture. Specifically, family-based studies have demonstrated that the development of a psychotic disorder increases the relatives' risk to develop the same or another psychotic disorder (Lichtenstein *et al.*, 2009; Van Snellenberg and de Candia, 2009; Dean *et al.*, 2010; Mortensen, Pedersen and Pedersen, 2010). Also, molecular studies have evidenced a shared genetic vulnerability between psychotic disorders (see section 1.5).

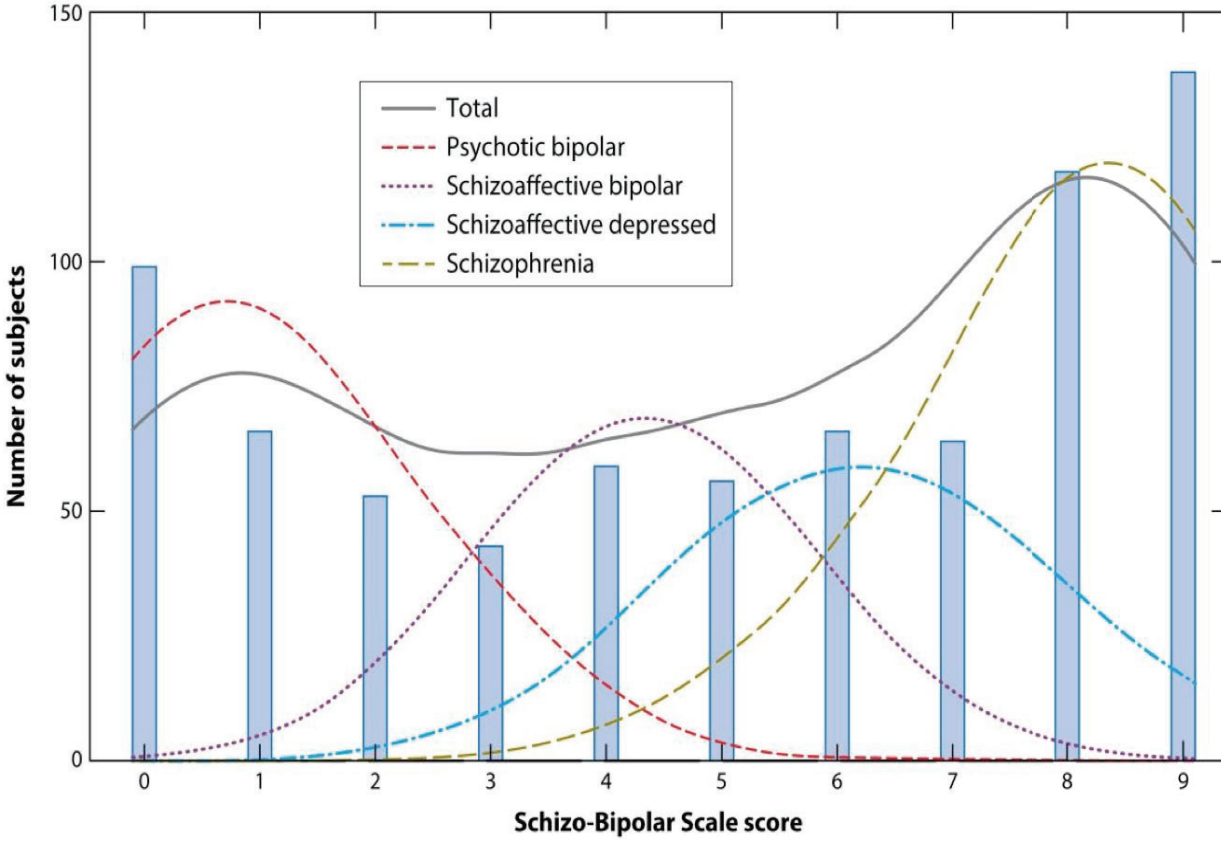


Figure 3. Graphical data supporting the continuous distribution of psychotic disorders. Keshavan et al. (2011) developed a new psychosis dimensional-scale that used both lifetime and cross-sectional symptom information to classify 762 cases diagnosed with schizophrenia, bipolar disorder and schizoaffective disorder. Each patient had a score that ranged from 0 (most bipolar-like value) to 9 (most schizophrenia-like value). As seen on the graph, while the majority of cases had ratings close to the prototypic schizophrenia or bipolar disorder diagnosis, a large group (45% of cases) fell on the continuum between these two diagnoses (schizoaffective disorder). Adapted from Pearlson (2015).

Along with clinical –and also aetiological– similarities among psychotic disorders, epidemiological studies support a continuity from subclinical symptoms to full-psychosis by showing that psychosis expression is present in the general population with a prevalence of 5-8% (van Os *et al.*, 2009; Linscott and Van Os, 2013; McGrath *et al.*, 2015; Nordgaard *et al.*, 2019). Similarly, clinical studies have shown clear resemblances in the clinical profiles of patients with psychotic symptoms and healthy individuals with subclinical psychotic symptoms, including personality traits such as schizotypy or subtle cognitive deficits (Claridge, 1997; Krabbendam *et al.*, 2004; Barrantes-Vidal, Lewandowski and Kwapil, 2010; DeRosse *et al.*, 2014; Mollon *et al.*, 2016). Also, as introduced in section 1.5, genetic studies have shown similarities in

the genetic background underlying both subclinical and clinical psychotic symptoms, suggesting again that psychotic expressions are distributed in the general population on a single continuum of vulnerability risk factors called the psychosis continuum (DeRosse and Karlsgodt, 2015). Accordingly, it is considered that the psychosis continuum reflect a continuous liability distribution in the general population, encompassing a full range of psychotic expressions from subclinical manifestations to the clinically significant symptoms observed in individuals diagnosed with a psychotic disorder, such as schizophrenia or bipolar disorder (van Os and Linscott, 2012; see **Figure 4**).

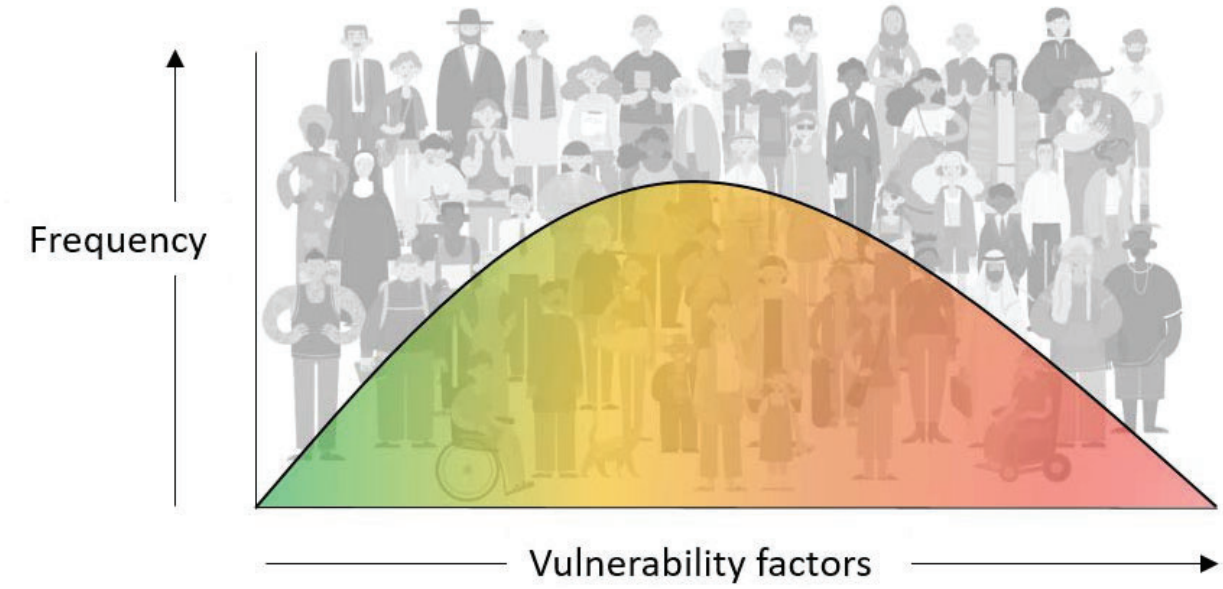


Figure 4. The psychosis continuum liability-threshold model. Assuming a normal distribution of the liability in the general population, the phenotypic outcome (here represented in different colours) might be determined quantitatively by the combined effects of vulnerability factors. If cumulative predisposition exceeds a certain threshold value, the individual manifests the clinical syndrome. Therefore, this model assumes that psychotic symptoms fluctuate in a population from a normal state of functioning (green), in individuals with none or few risk factors; going from subclinical psychotic experiences (orange) towards its clinical manifestation in the form of certain psychotic-spectrum disorders (red), as individuals have more risk factors.

According to this model, those individuals from the general population that do not have a psychotic disorder diagnosis but have some vulnerability factors and/or manifest some subclinical phenotypes such as schizotypy or low cognitive performance may be considered high-risk individuals. For example, unaffected

relatives of patients with schizophrenia are high-risk individuals because they share vulnerability risk factors, both genetic and environmental, with patients. Another group of at-risk individuals might be those healthy subjects who manifest high levels of subclinical phenotypes, such as schizotypy.

In agreement with the psychosis continuum framework, the present thesis has taken into account these at-risk phenotypes, and they have been analysed in family-based samples (with at least one subject with psychosis) as well as in general population-based samples.

1.4. Neurobiology of psychotic disorders: the neurodevelopmental model

The brain is the most complicated organ comprised of a large number of interconnections, and a correct formation is essential for its adequate functioning. Human brain development begins in the third gestational week and extends through at least late adolescence (Schmitt *et al.*, 2014; Teeuw *et al.*, 2018). It involves a dynamic sequence of processes, including neural cell differentiation, neuron production, migration and differentiation, myelination and sculpting of synaptic and circuitry architectures, that are under genetic control across the lifespan (Stiles and Jernigan, 2010; Catts *et al.*, 2013; Mills *et al.*, 2016; Teeuw *et al.*, 2018). In addition, these processes are also environmentally influenced, meaning that brain development is sensible to stressful events occurring during prenatal, early childhood and adolescent stages. Therefore, brain formation and maturation emerges as an ongoing dialogue between a child's genetic heritage and his or her environment (see **Figure 5**).

Despite the etiopathogeny[①] of schizophrenia is still unknown, the dominant hypothesis has been the neurodevelopmental one. It suggests that a disruption of brain development during early life underlies the later emergence of psychosis during adolescents or adulthood (Murray and Lewis, 1987; Weinberger, 1987; Rapoport, Giedd and Gogtay, 2012; Birnbaum and Weinberger, 2017). According to this model, both genetic and environmental inputs are involved in brain formation and

maturation and the variability or disruption of any of them could lead to dysfunctions of neurotransmission circuits that mature relatively late in adolescence (mainly the dopaminergic and glutamatergic systems; see **Box 1**). These alterations, in turn, might underlie the observed variability in brain-related phenotypes such as cognition, personality or affection across the psychosis continuum, from health to psychiatric disorders such as schizophrenia.

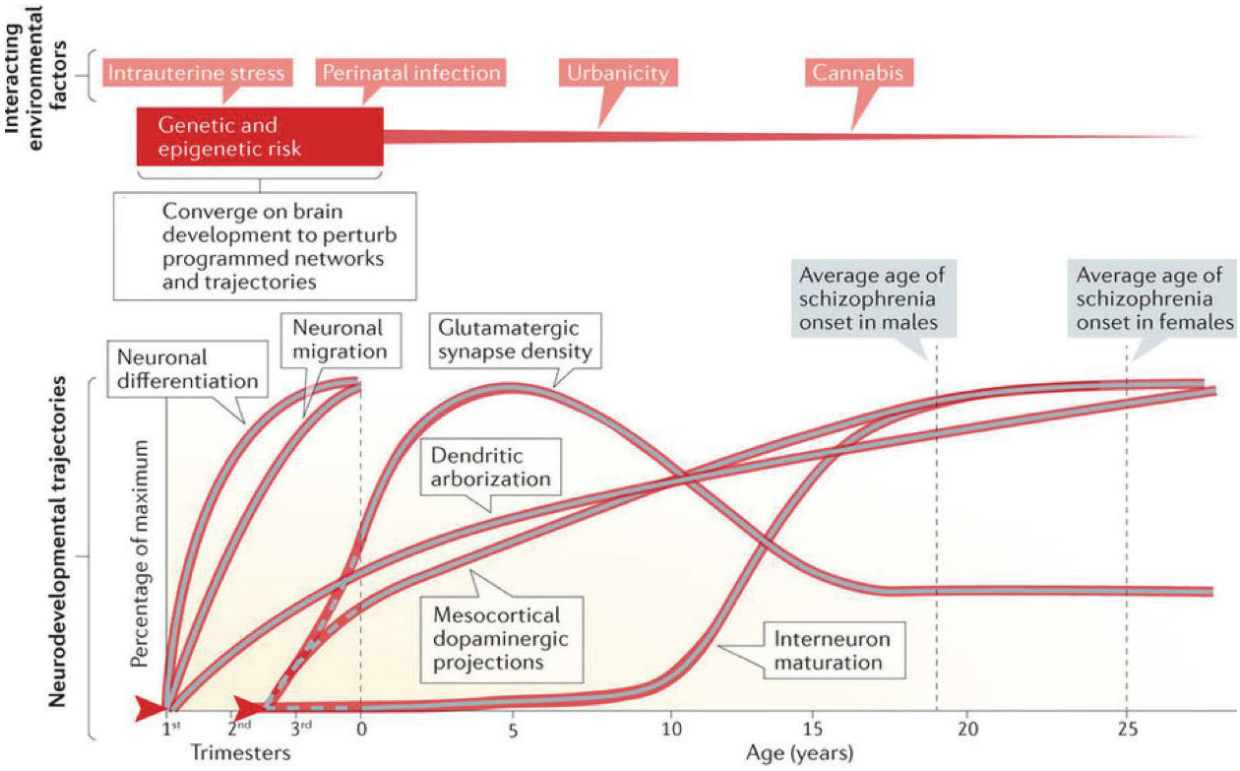


Figure 5. The neurodevelopmental model of schizophrenia. Schizophrenia risk genetic variants, in combination with each other and influenced by environmental risk factors, might disrupt different processes leading to an altered neurodevelopmental trajectory, culminating in a predisposition towards the dysfunction of neural circuits that mature late in adolescence (mainly prefrontal cortex circuits). In consequence, the shaped brain would be more vulnerable to the effect of new environmental stress factors (e.g. cannabis use), increasing the risk to develop schizophrenia later in life. Adapted from Birnbaum and Weinberger (2017).

Box 1. The dopaminergic and glutamatergic neurotransmission in schizophrenia

Strong evidence coming from pharmacological (Lieberman, Kane and Alvir, 1987; Krystal *et al.*, 1994; Lynch and Guttmann, 2001), post-mortem (Mackay *et al.*, 1982; Humphries *et al.*, 1996; Sokolov, 2002; Howes *et al.*, 2012), neuroimaging (Slifstein *et al.*, 2015; Kesby *et al.*, 2018) and genetic (Ripke *et al.*, 2014) studies suggest that deregulations in the dopamine and glutamatergic neurotransmission systems might underlie schizophrenia's pathophysiology. In this sense, it is believed that an excess of dopamine signalling in the striatal and/or mesolimbic areas of the brain might cause the positive symptoms of the disorder. In contrast, deficits in prefrontal cortical dopamine signalling might cause negative symptoms (Davis *et al.*, 1991). As dopamine and glutamatergic systems are interconnected, it is hypothesised that alterations in the glutamatergic system (mainly through a N-methyl-D-aspartate receptor, NMDAR, hypofunction) might underlie the unbalance of the dopamine circuits (Stahl, 2007).

The neurodevelopmental hypothesis of schizophrenia is supported by multiple sources of evidence from research fields as diverse as epidemiology, molecular biology, neuroimaging and genetics. Epidemiological studies have shown that individuals who later manifest schizophrenia report deviations from typical early childhood development (Rapoport, Giedd and Gogtay, 2012; Debnath, Venkatasubramanian and Berk, 2015) and have identified several maternal environmental factors to predispose offspring to psychosis, including exposure to infection, malnutrition in utero, preterm birth or low birth weight (Murray and Lewis, 1987; Weinberger, 1987; Belbasis *et al.*, 2017; Zwicker, Denovan-Wright and Uher, 2018). Molecular biology and neuroimaging studies have shown that the alterations in physiology, neurochemistry and brain structure and function typically documented in patients with schizophrenia are present prior to the onset of schizophrenia and evolve during the course of the disorder (Lawrie *et al.*, 2001; Pantelis *et al.*, 2003; Keshavan *et al.*, 2008; Jung *et al.*, 2010; Mechelli *et al.*, 2011; Tognin *et al.*, 2014; Niendam *et al.*, 2018). As an example, premorbid cognitive deficits are already present in children who later develop schizophrenia (Reichenberg *et al.*, 2010; Khandaker *et al.*, 2011; Sheffield, Karcher and Barch, 2018). Also, both individuals with schizophrenia and individuals at risk are more likely to present impaired neurodevelopment markers, including dermatoglyphic abnormalities, minor physical anomalies or neurological soft signs than general population individuals (Bramon *et*

al., 2005; Gabalda and Compton, 2010; Aksoy-Poyraz *et al.*, 2011; Theleritis *et al.*, 2012; Mittal *et al.*, 2014; Chan *et al.*, 2016, 2018; Radua *et al.*, 2018). This thesis has specifically focused on studying one of these neurodevelopmental markers, the dermatoglyphic abnormalities (see **Figure 6** and **Box 2**), and their relation with another schizophrenia vulnerability marker such as schizotypy, in a sample of families affected with a psychotic disorder.

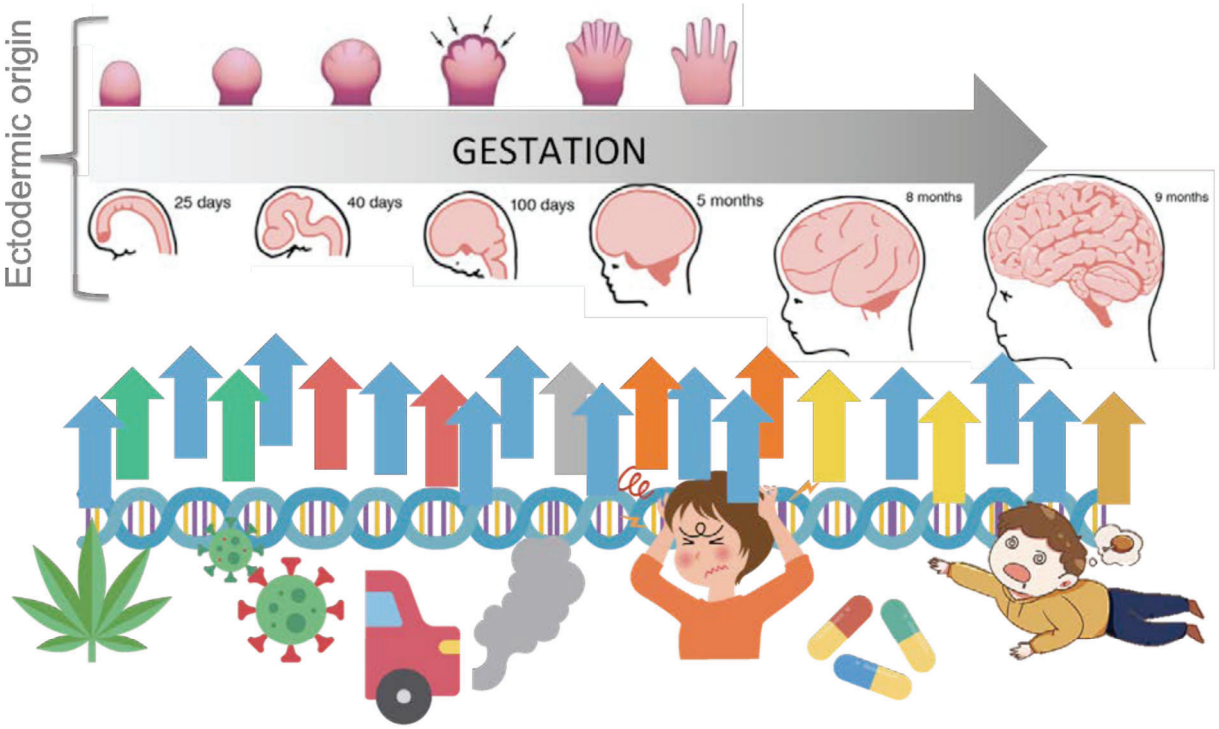


Figure 6. The dermatoglyphic pattern. The dermatoglyphic pattern is the epidermal ridge pattern that forms prints on the fingers, hands and soles in primates. Each individual has a unique dermatoglyphic configuration largely determined by genetic and intrauterine environmental factors. Once their formation is complete (24 weeks of gestation), the dermatoglyphic pattern remains unchanged over lifetime. Dermatoglyphics share an ectodermic origin with the central nervous system, and their formation co-occurs with crucial processes during the late first and second trimester of prenatal brain development (Rakic, 1988; Babler, 1991), causing that the alteration in one of these systems leads to the alteration of the other. Accordingly, deviations in dermatoglyphic patterns are considered potential etiopathogenic markers of schizophrenia risk by reflecting disruptions of gestational ectodermal development (Golembo-Smith *et al.*, 2012).

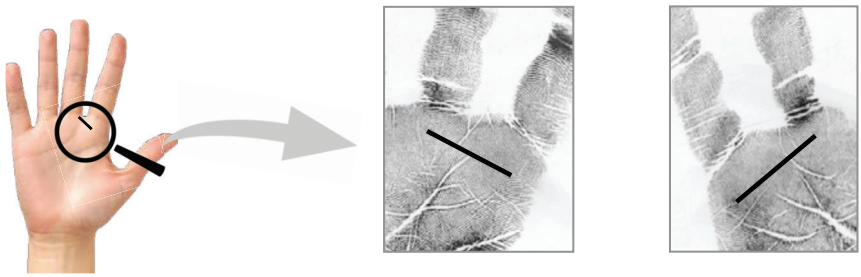
The neurodevelopmental hypothesis is also consistent with genetic studies (see section 1.5), which have revealed that many of the schizophrenia-associated genes are prenatally expressed and are involved in several neurodevelopmental processes, including neuronal differentiation, maturation or synapse formation (Birnbaum *et al.*,

2015; Skene, Roy and Grant, 2017; Consortium *et al.*, 2019). More recently, genetic-environmental interaction studies have also revealed the interplay between schizophrenia genetic risk variants and early-life complications (Ursini *et al.*, 2018, 2021).

Box 2. Dermatoglyphic measures of interest in psychiatric research

Dermatoglyphic measures can be classified as quantitative or qualitative (Cummins and Midlo, 1943) (Cummins and Midlo, 1943). Quantitative traits include the palmar a–b ridge count, which measures the number of ridges between the triradius a, in the base of the index digit, and the triradius b, in the base of the medium finger (as it is illustrated in the figure below); and the total a–b ridge count, which is defined by the sum of the right and left a–b ridge counts. Qualitative traits include short, broken segments of lines that cover the area with dermatoglyphic patterns in a disorganised way, which are termed ridge dissociations. The present thesis has particularly focused on ridge dissociations.

In comparison to healthy subjects, patients with psychotic disorders have a higher prevalence of reduced ridge counts (Fañanás, Moral and Bertranpetit, 1990; Fañanas *et al.*, 1996; Jelovac *et al.*, 1999; Fearon *et al.*, 2001; Bramon *et al.*, 2005) and ridge dissociations (Rosa, Fañanas, *et al.*, 2000; Rosa *et al.*, 2002), which are also more frequent in first-degree relatives of patients with schizophrenia (Fatjó-Vilas *et al.*, 2008). As vulnerability markers, it is interesting to note that dermatoglyphic alterations have also been associated with other schizophrenia liability markers, such as schizotypy, in both healthy relatives and controls (Rosa, van Os, *et al.*, 2000; Chok and Kwapil, 2005; Gabalda and Compton, 2010).



Left palmar a-b ridge count Right palmar a-b ridge count

1.5. Genetics of psychotic disorders

Despite schizophrenia etiological and pathophysiological underpinnings remain largely undetermined, there is considerable evidence to conclude that it is a complex phenotype resulting from both genetic and environmental etiological influences. To illustrate the importance of genetics, it is worth mentioning that a positive family history of schizophrenia is the strongest single indicator of an individual's schizophrenia risk (Mortensen, Pedersen and Pedersen, 2010).

The effect of familial and genetic influences on a disorder is estimated by family and twin studies. Family studies seek to analyse whether a condition of interest aggregates in families. In reference to psychosis, these studies have shown that the rate of schizophrenia is higher in relatives of patients with the disorder than in the general population (Henriksen *et al.*, 2017) and, more concretely, that the risk for developing the disorder depends on the increasing number of shared genes between family members and their affected relatives (Vogel and Gottesman, 1991; Lichtenstein *et al.*, 2006; see **Figure 7**). As a matter of fact, a recent meta-analysis has estimated that the risk of suffering schizophrenia among relatives with one affected proband is eight-fold compared to general population individuals (LE *et al.*, 2020). Interestingly, this vulnerability associated with the familial risk does not seem to be diagnosis-specific as relatives of patients with schizophrenia have increased risk for several psychiatric conditons, including bipolar disorder (Rasic *et al.*, 2014; Sandstrom *et al.*, 2020; Kendler *et al.*, 2021).

These findings show the familial nature of schizophrenia; however, they do not confirm a genetic over a familial environmental cause. In this context, twin studies are conducted to estimate the genetic and environmental contributions to the variance in liability to the disorder. Twin studies are based on the examination of the concordance rate of a phenotype in monozygotic and dizygotic twins. Monozygotic twins carry almost 100% identical genetic information, whereas dizygotic twins only share 50%, and a genetic contribution to disease is inferred when the concordance rate of a disease is higher in monozygotic twins. As schizophrenia concordance rates are 41%-65% in monozygotic twins and 0-28% in dizygotic twins (Cardno and

Gottesman, 2000), it is accepted that the familial aggregation of schizophrenia is mainly due to genetic factors. As a fact, the contribution of inherited genetic variants to schizophrenia (heritability[ⓘ]) is estimated to be 60-80% (Sullivan, Kendler and Neale, 2003; Lichtenstein *et al.*, 2009; Hilker *et al.*, 2017), suggesting that genetic and environmental factors operate hand-in-hand to increase the schizophrenia vulnerability risk. To note, other psychotic disorders have heritability rates similar to those estimated in schizophrenia (e.g. heritability of bipolar disorder is estimated to be around 58-93%, Kieseppä *et al.*, 2004; Song *et al.*, 2015).

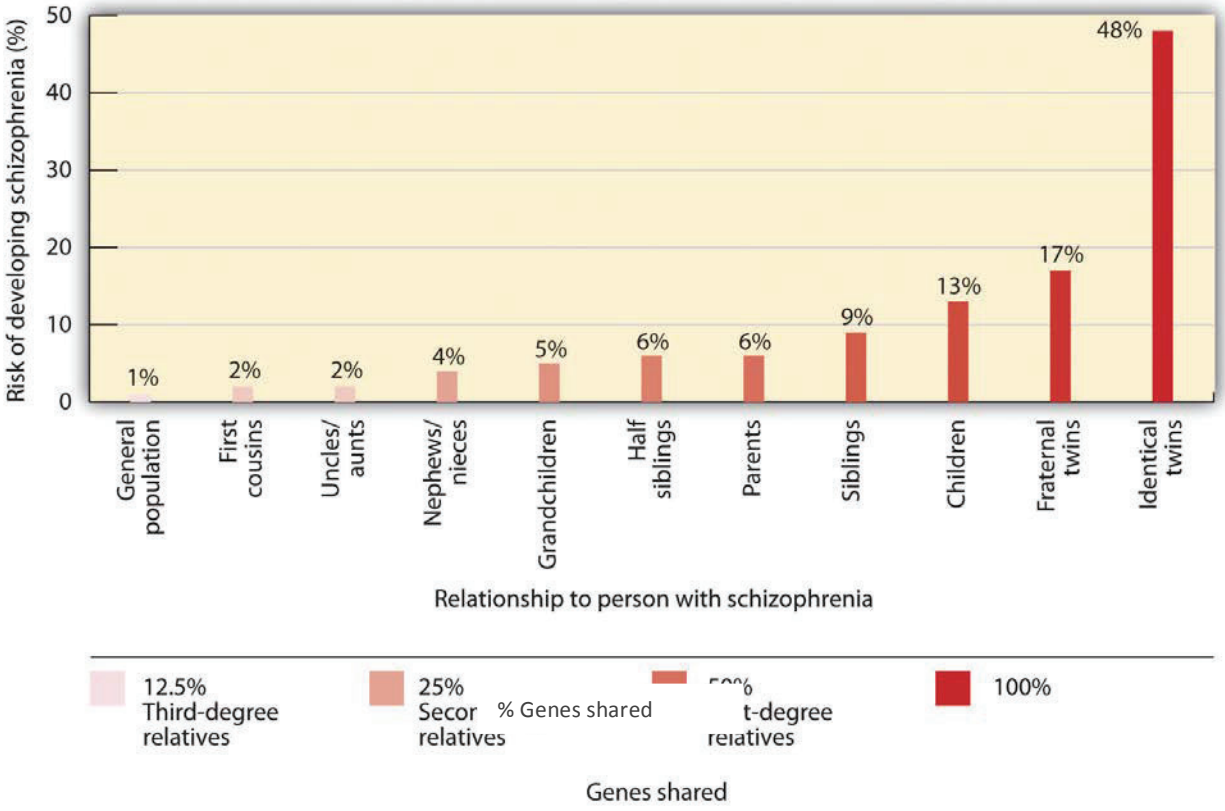


Figure 7. Rates of schizophrenia among relatives of patients with schizophrenia. As this graph reflects, the risk for developing schizophrenia depends on the increasing number of shared genes between family members and their affected relatives. Adapted from Vogel and Gottesman (1991).

In this regards, several decades worth of scientific research have revealed robust evidence regarding the genetic mechanisms underlying schizophrenia and other psychotic disorders. Nevertheless, according to the preeminent role of genetics in the pathophysiology of psychotic disorders, more studies are needed to elucidate the genetic architecture[ⓘ] underlying these disorders in order to understand their pathophysiology and, therefore to provide clinically helpful guidance for differential

diagnosis, treatment selection and/or novel treatments based on genetic mechanisms.

Genetic studies have revealed that psychotic disorders are highly polygenic. Accordingly, the genetic risk for schizophrenia is largely due to either the coincident inheritance of many common alleles of small effects (single nucleotide polymorphisms, SNPs[Φ]) and the presence of few rare mutations with a large effect (copy number variants, CNV[Φ] and single nucleotide variants, SNV[Φ]) (Bodmer and Bonilla, 2008; Kirov *et al.*, 2014; Geschwind and Flint, 2015; Kirov, 2015; Mulle, 2015; Tansey *et al.*, 2015; Marshall *et al.*, 2017; see **Figure 8**).

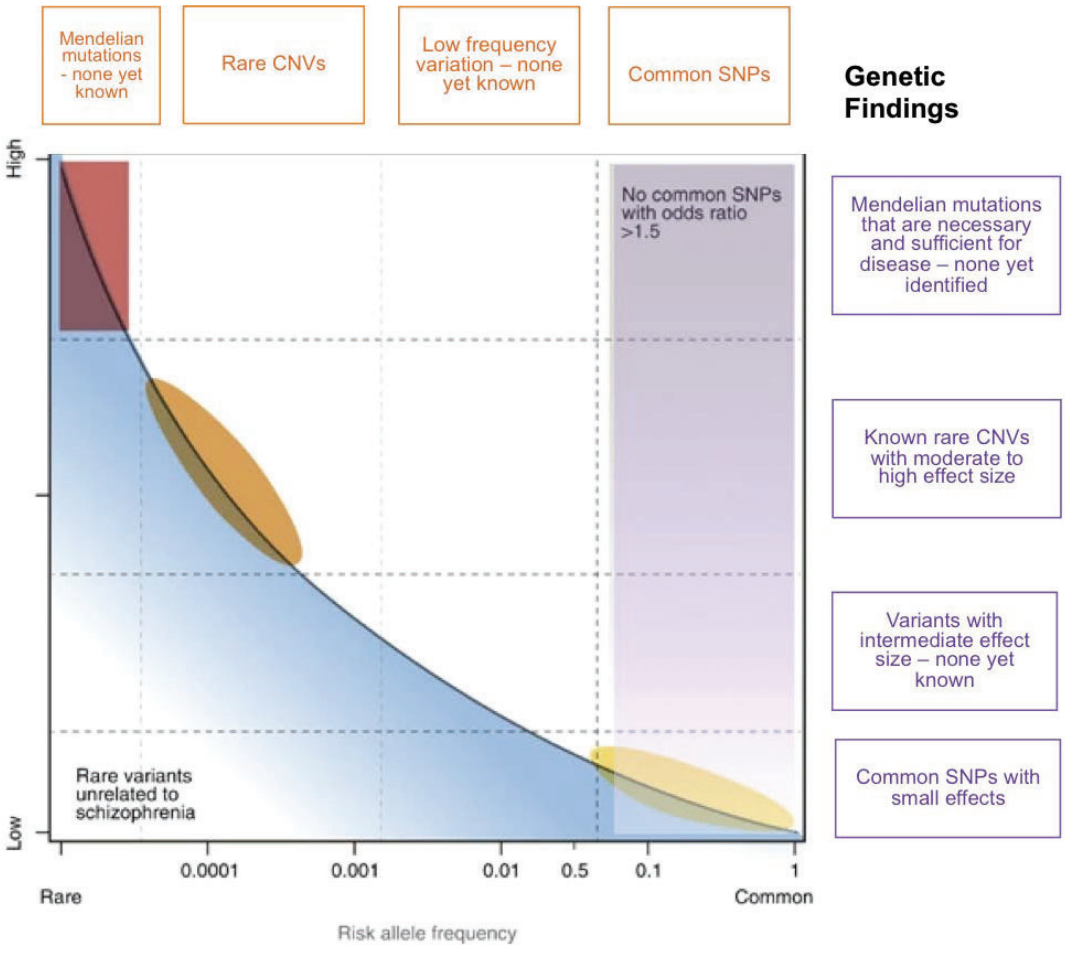


Figure 8. Representation of current genetic findings in schizophrenia. The effect size of identified risk alleles is roughly inversely proportional to allele frequency (black curve), although the strength of this relationship is unknown (blue shading). Common variation (SNPs) have a frequency of >1%, and each one confers a modest level of risk for the disorder (OR=1.1-1.5); rare mutations (CNV, SNV) have a frequency of <1% and have a larger penetrance on the phenotype (OR=2-41). Adapted from Mowry and Gratten (2013).

To illustrate this, the three largest genome-wide association studies (GWAS[ⓘ]) conducted so far in schizophrenia have identified 108 (Ripke *et al.*, 2014), 145 (Pardiñas *et al.*, 2018) and 270 (available as a pre-print, Ripke, Walters and O'Donovan, 2020) independent associated risk loci, respectively. Although these results are promising, the whole picture must be put into perspective. In this sense, it has been estimated that the aggregated effect of all the common variants associated with schizophrenia (polygenic risk score[ⓘ]) accounts for 7% of its phenotypic variance in case-control status (Ripke, Walters and O'Donovan, 2020), meaning that the set of known common variants "only" explains the 20-40% of schizophrenia's heritability (Lee *et al.*, 2013; Loh *et al.*, 2015; Speed *et al.*, 2017; Pettersson *et al.*, 2018; Speed and Balding, 2019). However, as it has happened in the study of other complex phenotypes, such as height and body mass index (Wainschtein *et al.*, 2019), as technology advance and more common and rare genetic variants are identified, it is expected that the heritability estimated by using genetic variants might be similar to the value accounted by twin studies.

Data coming from genetic studies have also revealed that genetic risk for psychosis is pleiotropic; meaning that the identified genetic variants that increase the risk for schizophrenia-spectrum disorders also influence the risk for other mental disorders (see **Figure 9**), including bipolar disorder (Purcell *et al.*, 2009; Lee *et al.*, 2013; Tesli *et al.*, 2014; Anttila *et al.*, 2016; Boies *et al.*, 2017; Witt *et al.*, 2017), major depressive disorder (Lee *et al.*, 2013; Anttila *et al.*, 2016; Witt *et al.*, 2017), autism-spectrum disorder (Lee *et al.*, 2013) or attention-deficit hyperactivity disorder (Hamshere *et al.*, 2013; Anttila *et al.*, 2016), and developmental traits in the general population, including low IQ, speech, fluency and verbal reasoning problems, social cognition alterations and social and behavioural problems (Germine *et al.*, 2016; Riglin *et al.*, 2017).

Pathway and gene set enrichment analyses have showed that most of the identified schizophrenia-associated genetic variants are particularly enriched in synaptic neurotransmission genes (post-synaptic density-related genes, glutamatergic-related genes, dopaminergic-related genes, voltage-gated calcium channel-related genes) and neurodevelop-mental related-genes (Walsh *et al.*, 2008; Xu *et al.*, 2012; Kirov *et*

al., 2012; Lips *et al.*, 2012; Purcell *et al.*, 2014; Ripke *et al.*, 2014; Fromer *et al.*, 2014; Hall *et al.*, 2014; Network and Pathway Analysis Subgroup of Psychiatric Genomics Consortium, 2015; Gatt *et al.*, 2015; Singh *et al.*, 2016; Genovese *et al.*, 2016; Marshall *et al.*, 2017; Drakesmith *et al.*, 2018; Radulescu *et al.*, 2018; Ruderfer *et al.*, 2018; Schijven *et al.*, 2018; Ma *et al.*, 2018; Consortium *et al.*, 2019; Schork *et al.*, 2019; Howell and Law, 2019). These results are in accordance with the neurodevelopmental hypothesis of schizophrenia that suggests that individuals with schizophrenia carry genetic risk variants that impact brain development, resulting in disturbed neuronal communication that, later in life, might lead to the manifestation of a wide range of clinical and subclinical symptoms (see section 1.4).

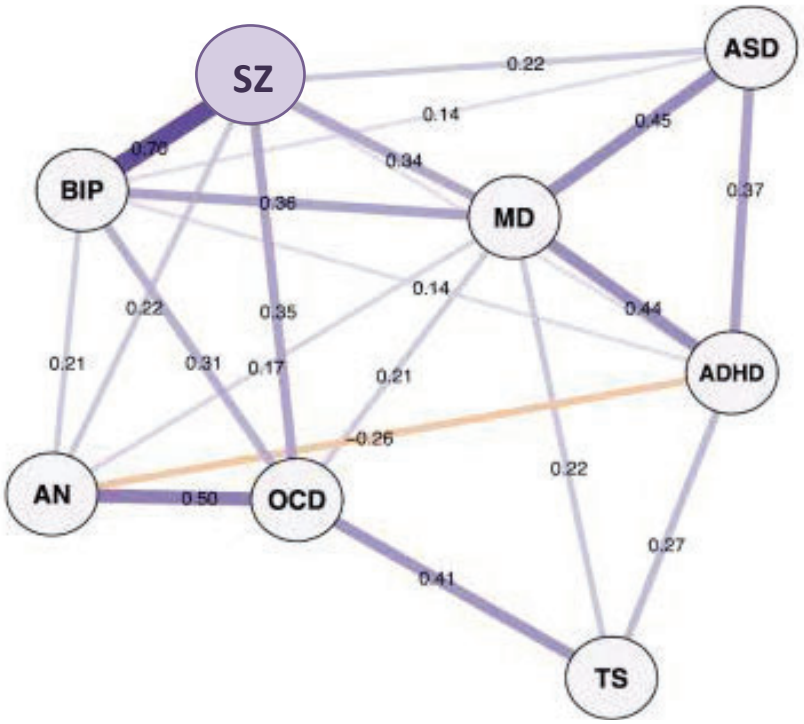


Figure 9. Genetic relationships between eight psychiatric disorders. SNP-based genetic correlations between eight disorders were depicted to reveal complex genetic relationships. Each node represents a disorder, with edges indicating the strength of the pairwise correlations. The width of the edges increases, while the length decreases, with the absolute values of correlations. As an example, it has been estimated that around the 70% of the common genetic variants associated with schizophrenia are also associated with bipolar disorder. ADHD: attention-deficit hyperactivity disorder, AN: anorexia nervosa, ASD: autism-spectrum disorder, BIP: bipolar disorder, MD: major depressive disorder, OCD: obsessive-compulsive disorder, SZ: schizophrenia, TS: Tourette syndrome. Adapted from Consortium *et al.* (2019).

Based on these data, the present thesis has focused on studying whether particular genes involved in synaptic transmission and neurodevelopment are associated with the expression of different vulnerability markers related to psychosis, including schizotypy and cognitive performance (see **Box 3**).

Moreover, as depicted in **Figure 9**, genetic studies have revealed that genetic variants that increase the risk for psychotic disorders also increase the risk for other neurodevelopmental disorders such as autism-spectrum disorders. A specific group of genes that have been associated with both pathologies are those encoding for scaffolding proteins, a type of proteins involved in the correct functioning of synapses. Accordingly, the present dissertation also includes a systematic review article in which the implication of several scaffolding genetic variants in the neurobiology of schizophrenia and autism-spectrum disorders was analysed.

Box 3. List of genes included in the present thesis

- D-amino acid oxidase activator, ***DAOA*** (13q33.2) encodes the protein DAOA, which activates D-amino acid oxidase (DAAO) in the brain, an enzyme that oxidises D-serine, an important co-agonist for the N-methyl-D-Aspartate receptor (NMDAR) (Chumakov *et al.*, 2002). Genetic studies indicate that *DAOA* is involved in the pathophysiology of psychotic disorders (Liu *et al.*, 2019). Expression studies have shown that subjects with a diagnose of schizophrenia have lower D-serine levels in serum (Hashimoto *et al.*, 2003) and cerebrospinal fluid (Hashimoto *et al.*, 2005) than healthy controls, while increased DAOA expression has been reported in the pre-frontal cortex (Korostishevsky *et al.*, 2004).

- Regulator of G-protein signalling 4, ***RGS4*** (1q32.2), which is expressed in the neocortex (Erdely *et al.*, 2004), is a member of a protein family that plays a crucial role in modulating signalling through G-protein pathways and acts as a GTPase activator by accelerating the hydrolysis of the guanine triphosphate (GTP) to guanine diphosphate (GDP). This reaction shortens the signal transduction duration of many neurotransmitters involved in psychotic disorders, such as dopamine or glutamate (Erdely *et al.*, 2006). Besides the genetic association with psychotic disorders (Chen *et al.*, 2004), expression studies have shown decreased RGS4 protein levels in the prefrontal cortex of individuals with schizophrenia compared to healthy subjects (Mirnics *et al.*, 2001).

- Zinc finger protein 804A, ***ZNF804A*** (2q32.1), which is expressed throughout the foetal and adult human brain (Hill and Bray, 2012; Tao *et al.*, 2014), has been repeatedly associated with psychosis (Riley *et al.*, 2010; Ripke *et al.*, 2011; Gratten *et al.*, 2014). Despite its exact functions remain unclear, molecular, and bioinformatics studies suggest that *ZNF804A* might acts as a putative transcription factor that plays pivotal roles in cell physiology, neurodevelopment regulation (Riley *et al.*, 2010) and synaptic plasticity (Hess and Glatt, 2014; Deans *et al.*, 2016). Interestingly, some of the genes regulated by *ZNF804A*, such as the Dopamine Receptor D2 (*DRD2*) or Catechol-O-Methyltransferase (*COMT*), are directly involved in dopaminergic transmission and have been associated with schizophrenia (Girgenti *et al.*, 2012). Therefore, current evidence suggests that deregulation of *ZNF804A* could contribute to the altered neuronal and synaptic structures that are associated with psychotic disorders.

- Akt serine/threonine protein kinase 1, ***AKT1*** (14q32.32) encodes a serine/threonine kinase (Akt serine/threonine protein kinase 1, Akt1) involved in modulating synaptic dopaminergic transmission systems, where it is key signalling intermediate downstream of dopamine receptor D2 (DRD2) (Scheid and Woodgett, 2001, 2003; Beaulieu *et al.*, 2005). According to this role, and taking into account that optimal execution of cognitive tasks critically depends on proper levels of dopamine within the prefrontal cortex, it has been suggested that differential *AKT1* gene expression could modulate cognition through the regulation of dopaminergic neurotransmission. In this sense, genetic variation within this gene has been associated with schizophrenia (Schwab *et al.*, 2005; Bajestan *et al.*, 2006; Karege *et al.*, 2010) and also with cognitive performance (van Winkel *et al.*, 2011; Ohi *et al.*, 2013; Klaus and Pennington, 2019).

1.6. Environmental factors associated with psychotic disorders

As introduced in section 1.5, twin studies suggest that environmental influences explain around 20% of the variance in liability to schizophrenia. Importantly, epidemiological studies have identified many environmental factors associated with the increased risk of schizophrenia (see **Figure 10**).

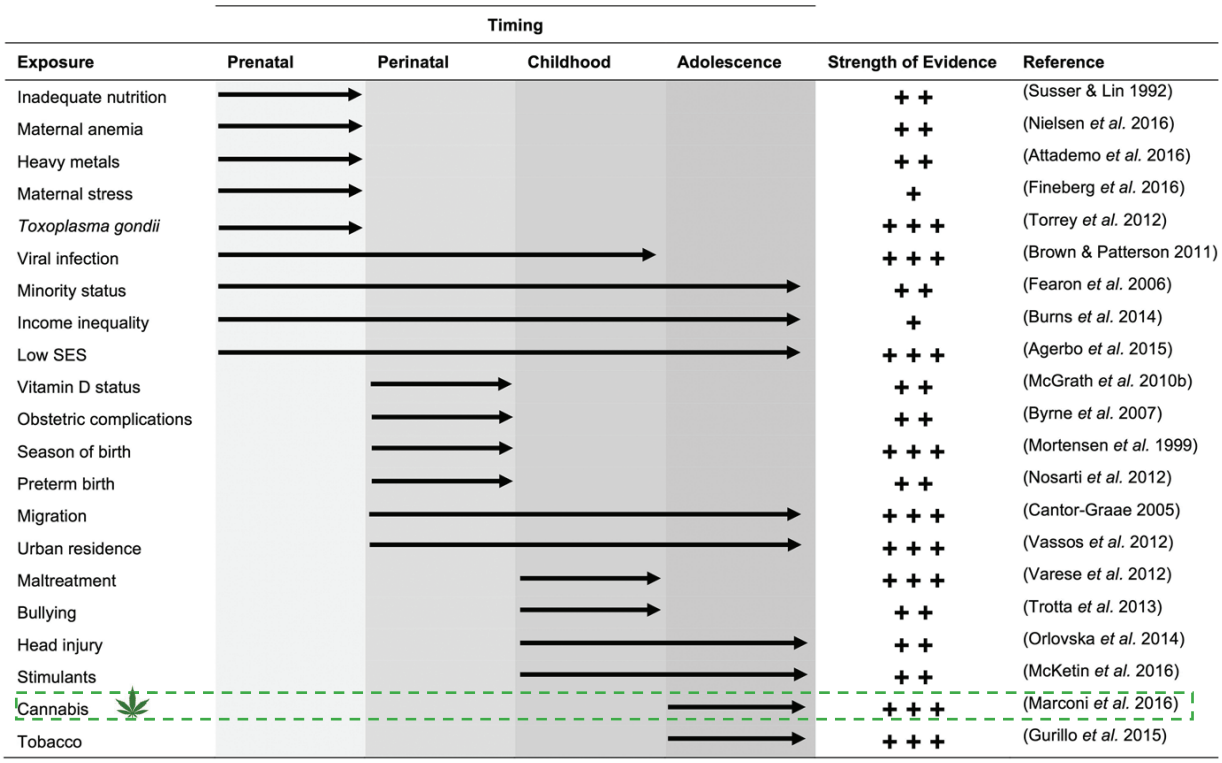


Figure 10. Environmental factors associated with psychosis. There are factors, such as low socioeconomic status (SES), that tend to remain constant throughout development. In contrast, other factors, such as cannabis use, might contribute to increase the risk of schizophrenia by their exposition during sensitive periods in the development. The number of plus signs denotes the strength of evidence for the association: +++ indicates consistent evidence from multiple large-scale studies or a meta-analysis; ++ indicates evidence from multiple smaller studies or a strong association in a high-quality study; + indicates evidence from a single study, multiple small/low-quality studies, or few studies with conflicting reports. Adapted from Zwicker, Denovan-Wright and Uher (2018).

The current knowledge on the environmental causation of psychosis suggests a complex picture with a multitude of social, physical and chemical exposures occurring at different stages of life, affecting the risk for schizophrenia and other psychotic disorders. As a complex phenotype, the risk of developing psychosis increases with the accumulation of many genetic risk variants and exposures to multiple adverse

environmental factors. In this context, there have been attempts to calculate an environmental risk score (ERS) to measure the aggregated effect of different environmental risk factors, analogous to what PRS achieves with genetic variation (Vassos *et al.*, 2018; Gillett, Vassos and Lewis, 2019). Although this marker is still in early development, it explains an estimated 7% of this variability in liability to psychosis (Gillett, Vassos and Lewis, 2019).

As explained in section 2.3, the present thesis has focused on investigating one specific environmental factor: the effect of the use of cannabis on the risk for psychosis.

2. Approaches to advance in the knowledge of psychotic disorders

As explained in section 1, psychotic disorders are heterogeneous phenotypes caused by the aggregated effect of multiple genetic risk variants and the influence of several environmental factors, including the use of cannabis (Purcell, 2002; Furrow, Christiansen and Feldman, 2011; Trerotola *et al.*, 2015; Merikangas and Merikangas, 2019). The genetic architecture of psychotic disorders is polygenic and pleiotropic (see **Figure 11**), and the study of the interplay between genetic and environmental risk factors is necessary to understand the aetiology[ⓘ] and pathogenesis[ⓘ] of these conditions and, ultimately, to develop more effective treatments (Fabbri and Serretti, 2020).

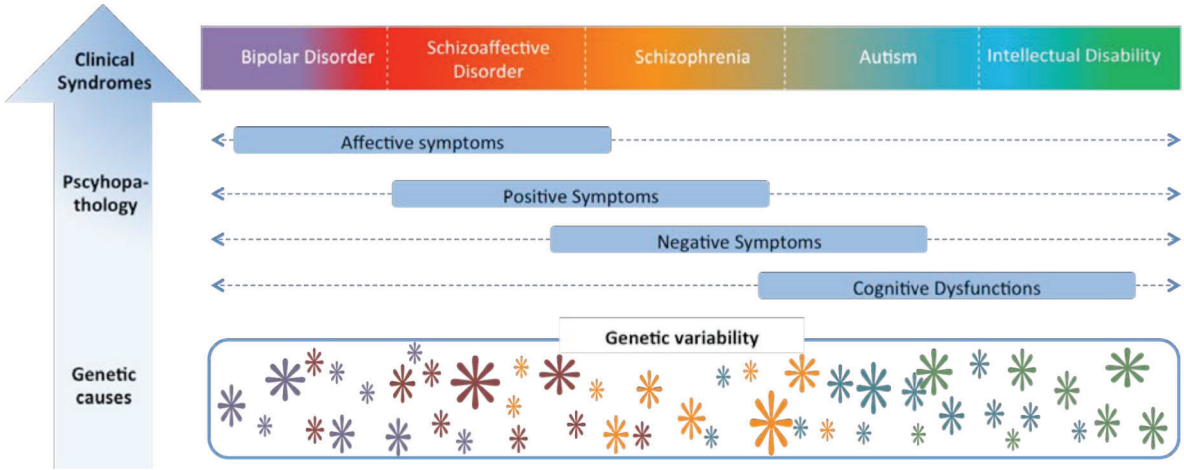


Figure 11. The hypothesised continuum model of the complex relationship between genetic variation and clinical phenotypes. This conceptual model shows relationships between genotype and clinical phenotype, starting at genetic variation (the bottom tier of the figure). Genetic variants are represented by asterisks with different effect-sizes and contribute to the different psychopathology domains and clinical and subclinical symptoms (e.g. schizotypy, cognitive dysfunction) present across the psychosis continuum. Adapted from (Pearlson, 2015).

Despite the advances in this field, we still have much room for improvement in mapping genetic variants and biological pathways to the phenotypic heterogeneity within schizophrenia and other psychotic disorders. As individuals with psychotic disorders show a wide range of different symptoms –probably due to diverse genetic background (Coombes *et al.*, 2020)– the relative representation of the underlying

genetic variants might vary between patients. However, lacking better alternatives, most studies to date have tended to treat schizophrenia and other psychotic disorders as unitary disease entities. This strategy might difficult the identification of genetic variants and biological mechanisms that give rise to psychotic disorders, as they may not be the same for all individuals. Therefore, if we want to improve the efficiency of the genetic association analyses in complex traits such as schizophrenia or bipolar disorder, we need first to overcome their clinical heterogeneity (van der Sluis *et al.*, 2010; Manchia *et al.*, 2013; Liang and Greenwood, 2015; Narita *et al.*, 2020).

Different strategies might help to unravel the phenotypic heterogeneity of psychotic disorders and to better map the genotype-phenotype underlying these disorders. Among these strategies, the present thesis proposes using family-based designs and intermediate phenotypes to facilitate the identification of genetically more homogeneous forms of psychotic disorders and the conduction of gene-environment studies to advance our knowledge of causal mechanisms leading to psychosis and psychosis-associated phenotypes, including schizotypy and cognitive performance.

2.1. The use of family-based studies

As introduced in section 1.5, a positive family history of schizophrenia still remains as the most important risk factor for developing the disorder (Mortensen, Pedersen and Pedersen, 2010; Chou *et al.*, 2017). This makes sense as individuals of the same family share more genetic and environmental vulnerability factors than unrelated persons. In addition, and under the psychosis continuum paradigm (see section 1.3), relatives of patients with schizophrenia tend to show an increased prevalence of subclinical symptoms, including schizotypy and cognitive deficits (Schulsinger, 1976; Kendler *et al.*, 1993; Tienari *et al.*, 2003; van Os and Linscott, 2012; van Os and Guloksuz, 2017).

Based on these data, the present thesis proposes using family-based samples with informative clinical data as a helpful strategy to reduce the clinical heterogeneity and the genetic complexity of psychotic disorders. Specifically, we have focused on two different and complementary approaches based on family-based samples in order

to, first, identify more homogenous forms of a disorder at a family-level (familial aggregation approach), and, second, to facilitate the subsequent identification of underlying genetic factors (family-based association approach).

2.1.1. The Familial aggregation approach

A valuable strategy to guide the stratification of families in order to reduce heterogeneity and to facilitate the identification of genetically more homogeneous forms of a disorder is the analysis of the familial aggregation or familiality of a trait; which measures the phenotypic resemblance among family members (Peralta *et al.*, 2015; Walsh *et al.*, 2020; Iorfino *et al.*, 2021). A trait is familial when family members are more similar for this trait than they would be if they were not related. In this regard, the familial aggregation of a trait can be understood as the clustering of a trait (e.g. schizotypy) within a given family, which is usually caused by genetic or environmental similarities and can be estimated using different approaches (see **Box 4**).

Box 4. Main approaches to estimate the familial aggregation of a trait

- The most common approach is by means of the **intraclass correlation coefficient (ICC)**, which measure the strength of familial aggregation for a trait in a sample of families. The ICC ranges from 0 to 1, and when it is significantly superior to 0 it means that the trait aggregates in the sample. For example, schizotypy is considered a phenotype with a moderate familial aggregation (ICC=0.27) (Vassos *et al*, 2008).

- A second approach to estimate the familial aggregation of a trait is comparing **the levels of the trait between patients, relatives and healthy controls**. Continuing with the same example, comparison studies show that patients tend to present higher schizotypy levels than healthy controls and that relatives have intermediate levels (Shah *et al*, 2015).

- A third approach to analysing a trait's familial aggregation o is the study of the **correlation of this trait between pairs of first-degree relatives (proband-sibling or proband-parent)**. These studies have also reported schizotypy familiality (Clementz *et al.*, 1991; Grove *et al.*, 1991; Berenbaum and McGrew, 1993).

The estimation of familiality has been successfully used to detect those phenotypes that maximise the phenotype-genotype correlation as a first step in unravelling the molecular genetic underpinnings of psychotic disorders (Peralta et al., 2015). As an example, this strategy has been used to dissect the heterogeneity of the major depressive disorder, where age at onset was found to significantly aggregate in families (Ferentinos et al., 2015). Another example comes from a study that examined the familiality of catatonia in a sample of families with psychotic disorders. They found that some catatonia-related phenotypes exhibited substantial familial aggregation, suggesting that they are appropriate phenotypes for subsequent molecular studies (Peralta *et al.*, 2017). With a similar approach, Bigdeli et al. (2020) observed that the familial architecture of neurocognitive functions appears to be different in childhood-onset schizophrenia than in adult-onset schizophrenia families. In adult-onset families, there were significant shared familial effects on attention and working memory, whereas in childhood-onset ones, there were significant shared familial effects on verbal learning and memory for faces.

These examples illustrate how the estimation of the global degree of familiality of a phenotype might improve the identification of vulnerability markers for psychotic disorders that could posteriorly be used in genetic association studies to identify risk variants for the disorders. However, analogous to what happens with individuals, clinical and subclinical profiles of families affected by psychotic disorders are also heterogeneous. By following the schizotypy example, a sample of families with at least one patient diagnosed with a psychotic disorder could be comprised of families whose all members show high levels of schizotypy, other families whose all members show low schizotypy levels and a group of families with heterogeneous levels (this is, some members show high and others show low levels of schizotypy). In order to better manage the phenotypic heterogeneity between families, it would be advantageous to stratify and distinguish subgroups of families according to their shared level of vulnerability for the disorder. If carried out, this strategy could contribute to analyse the genetic complexity of psychotic disorders by facilitating the identification of families with different liability factors for a disorder (i.e. defining different aetiological subgroups). Accordingly, one of the aims of the current thesis was to develop a continuous score (intrafamilial resemblance score, IRS) to

quantitatively estimate the similarity of a trait among family members (in each family) so that a set of families could be classified according to their phenotypic similarity (see **Figure 12**).

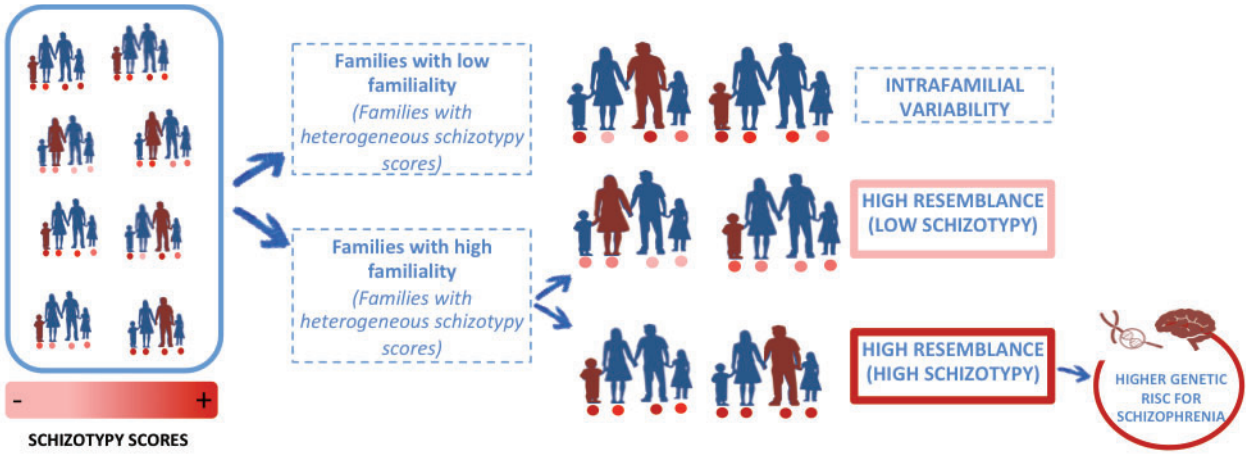


Figure 12. The intrafamilial-resemblance score (IRS) approach. In this thesis, we aimed to develop a method that might allow identifying different aetiological subgroups of psychosis. As represented in the figure, and continuing with the previous example of schizotypy, this strategy would allow to focus on each family as a unit of study and to calculate a continuous score (IRS) to estimate the similarity of schizotypy levels among family members, so that we could distinguish "low familiality" families (all members show discordant levels of schizotypy) from "high familiality" families (all members show similar levels of schizotypy, either low or high). The ultimate goal of this strategy would be identifying those families with a higher genetic liability for psychosis (those families whose members share a similar high level of schizotypy).

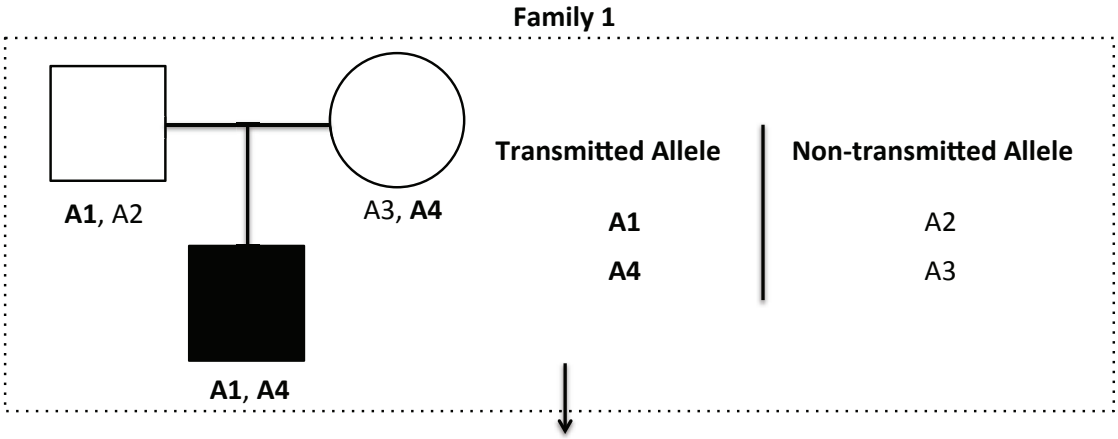
2.1.2. The family-based association approach

When a particular phenotype proves to be familial; this is, to aggregate in families, it is assumed that genetic factors make substantial contributions to the trait. Accordingly, genetic association studies can be conducted to test the correlation between the phenotype and genetic variation to identify candidate genes that contribute to the trait.

The most common design to test genetic associations is the case-control study, which compares the genotype or allele frequency at a putative locus between affected subjects and unaffected controls and test whether there is a significant difference between the two populations. Particular case-control studies in which

hundreds of thousans of genetic variants are tested are GWAS. As GWAS are conducted in large samples, a careful selection of individuals is necessary to ensure homogenous genetic background and avoid possible population stratification[ⓘ]. Also, due to the need to collect large samples with a same diagnosis, GWAS tend to neglect the phenotypic differences observed between cases, which are crucial for mapping the heterogeneous disease phenotype at the individual level (Foulkes and Blakemore, 2018; Wolfers *et al.*, 2018).

In contrast to case-control designs, family-based studies are genetic association studies that avoid problems of population heterogeneity because all individuals in a family pedigree share a common genetic background and tend to be more homogeneous in exposure to environments associated with the disorder (Ott, Kamatani and Lathrop, 2011). Although their use have decreased in the last decades –mainly due to the difficulty to accumulate large enough samples of well-characterized families– the value of family-based association studies remains beyond question (Glahn *et al.*, 2018). The most common analysis conducted in family-based samples is the transmission disequilibrium test (TDT), which compares the frequency of a particular allele transmitted from a heterozygous parent to an affected offspring to the frequency of the allele that is not transmitted. In other words, it tests whether heterozygous parents transmit a particular genetic variant to affected offspring more frequently than expected by chance (see **Figure 13**). In this regard, the present thesis has used the TDT analysis to assess the involvement of genetic variation in the expression of clinical and subclinical symptoms.



The same analysis is conducted with N families

Alleles	% transmission	% non-transmission
A1	50	50
A2	50	50
A3	50	50
A4	70	30
A5	50	50
A6	50	50

Figure 13. The transmission disequilibrium test (TDT). TDT is performed on families with an affected child (black square). The allele transmitted from heterozygous parents to the affected child is counted. In this example, the child from family 1 has received the allele A1 from his father and the allele A4 from his mother. The same analysis is conducted with n families, and the % of transmission and non-transmission of each allele is counted. Under no association with the disease or trait, one would expect the parents' allele would be transmitted randomly to the offspring (50% of the time). However, if a particular allele increases the risk of disease or trait, this allele will be associated with a transmittion rate above the expected by chance (50%). In this example, the allele A4 is over-transmitted to the affected child (the affected child have inherited the A4 allele more times than expected) and thus, it is considered a risk allele for the disorder. Adapted from Giordano (2005).

2.2. The use of intermediate phenotypes

Psychotic disorders are complex phenotypes and genetic variation itself does not directly cause the altered behaviour observed in patients with psychosis (e.g. no gene encodes for hallucinations). Instead, genetic variation impacts neurobiological processes at multiple levels during development, affecting the brain function and structure and resulting in the psychopathological manifestations of the disorder. According to this, it has been proposed that the assessment of more reliable

quantifiable measures or traits related to specific neurobiological functions instead of categorical diagnoses, might enhance the identification of the underlying genetic variants (Gottesman and Gould, 2003; Manchia *et al.*, 2013; Greenwood *et al.*, 2019). These quantifiable markers are often referred to in psychiatric literature as intermediate phenotypes or endophenotypes (Lenzenweger, 2013). Any quantifiable trait associated with psychosis is considered an intermediate phenotype related to this clinical outcome when it is heritable, primarily stable and state-independent (meaning that it is manifested in an individual whether or not the disorder is active). In addition, it has to co-segregate with psychosis in families and has to be found in unaffected family members at higher rates than in the general population (Gottesman and Gould, 2003). As intermediate phenotypes do not only emerge symptomatically but are also present earlier in life, they are considered potential vulnerability markers for a disorder. A great variety of intermediate phenotypes have been associated with psychosis, including cognitive (e.g. attention, working memory, executive function, memory), personality (e.g. schizotypy, openness to experience), neurophysiological (e.g. prepulse inhibition of the startle response, P50 suppression), neuroanatomical (e.g. total brain volume, white brain matter connectivity), neurofunctional (fMRI, EEG) and neurological measures (e.g. neurological soft signs) (Turetsky *et al.*, 2007; Allen *et al.*, 2009; Greenwood *et al.*, 2011, 2016; Light *et al.*, 2014; Swerdlow, Gur and Braff, 2015; Cao *et al.*, 2016; Owens *et al.*, 2016; Birur *et al.*, 2017), among others.

The inclusion of intermediate phenotypes in research seeks to move away from the study of patients grouped through diagnoses and deconstruct these disorders into the contributing behavioural traits or phenotypes and their underlying neurological circuits and systems. Accordingly, the use of intermediate phenotypes contributes to the dissection of the clinical heterogeneity of psychosis by means of different strategies. First, their use might help to understand the aetiology of psychotic disorders by identifying individuals with a putative vulnerability for developing the disorder. For example, if a particular phenotype (e.g. schizotypy) is considered an intermediate phenotype or a vulnerability marker for schizophrenia, individuals with high schizotypy levels might be at risk for developing the disorder. Second, intermediate phenotypes can be used to identify subgroups of patients in terms of genetic aetiology (Meda *et al.*, 2016), which posteriorly facilitates the identification of

the underlying genotype-phenotype correlations and the development of new treatments (Wadhera *et al.*, 2016; see **Figure 14**). Moreover, one of the strengths of working with intermediate phenotypes is that they can also be measured, to a greater or lesser degree, in healthy individuals who are therefore free from the presence of confounding factors related to the disorder such as treatment (MacDonald *et al.*, 2009).

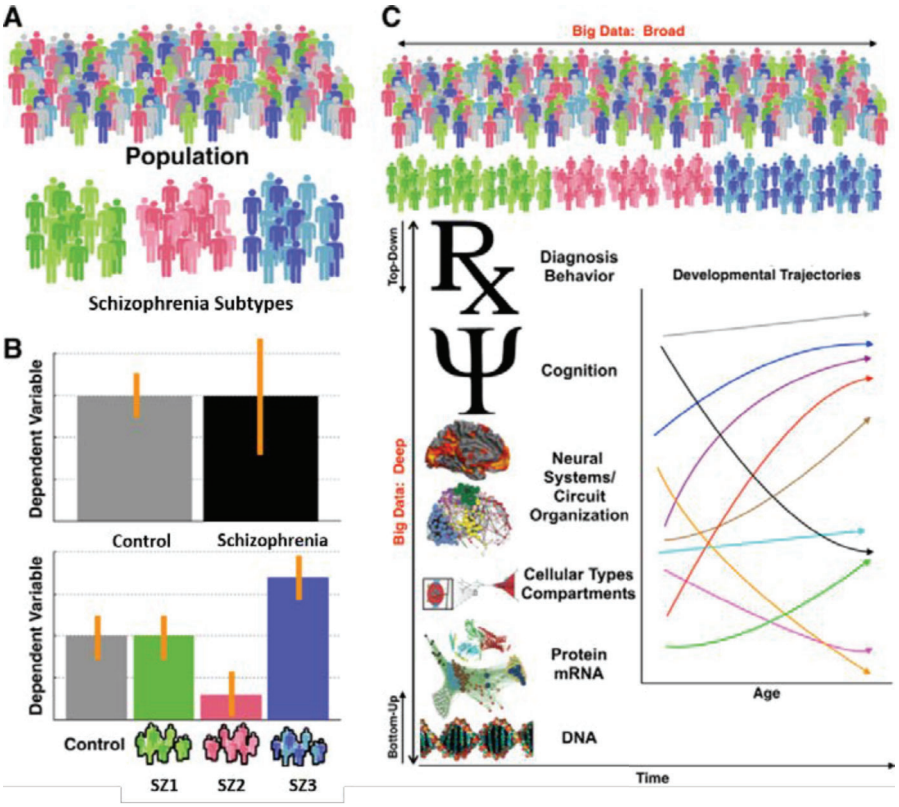


Figure 14. Approaches to decomposing heterogeneity in schizophrenia. (A) A population of interest is shown, and schizophrenia cases are coloured. The different colours represent different forms of schizophrenia. **(B)** With a typical case-control study, when patients with a diagnose of schizophrenia are compared to controls on some dependent variable, schizophrenia's heterogeneity is ignored. In this scenario, there is no clear case-control difference, but the schizophrenia group shows higher variability (indicated by the larger error bars). An approach towards decomposing heterogeneity might be to construct a stratified model whereby we model intermediate phenotypes instead of one schizophrenia diagnosis and then re-examine differences on the hypothetical dependent variable of interest. **(C)** Heterogeneity is shown in schizophrenia as multi-level phenomena. This panel also shows how development is another important dimension of heterogeneity to consider at each level of analysis. Adapted from Lombardo, Lai and Baron-Cohen (2019).

The present thesis has benefited from the use of two intermediate phenotypes associated with psychosis such as schizotypy and cognitive performance. On the one hand, a family-based sample was used to assess whether these phenotypes aggregated in families and, if so, whether they could be used to identify more homogeneous forms of psychotic disorders. On the other hand, these two vulnerability markers for psychosis were measured in non-clinical individuals in order to study the role of genetic and environmental factors on the risk for psychosis. The relationship between schizotypy and cognitive performance with psychosis is briefly described below.

2.2.1. Schizotypy

Schizotypy is a set of personality traits encompassing behaviours, cognitions and emotions that resemble the signs and symptoms of schizophrenia in the general population (Raine, 2006). It is comprised of three factors (cognitive-perceptual, interpersonal and disorganised factor) that correspond with the three main clusters of symptoms in schizophrenia (positive, negative and cognitive/disorganised symptoms). The cognitive-perceptual factor includes magical thinking, unusual perceptual experiences, ideas of reference and paranoia; the interpersonal factor includes constricted affect, social anxiety, lack of close personal relationships and suspiciousness, and the disorganised factor includes odd behaviour and odd speech (Debbané *et al.*, 2015).

Epidemiological studies have shown that levels of schizotypy are continuously distributed throughout the population, ranging from low levels and psychological health to extremely high levels and potential dysfunction in the form of psychosis (Claridge, 1997; Nelson *et al.*, 2013). Although present in the entire population, schizotypy meets the requirement of any schizophrenia-related intermediate phenotype. It is a familial trait with an estimated heritability of 50% (Linney *et al.*, 2003), and patients with schizophrenia (Chapman, Chapman and Raulin, 1978; Brosey and Woodward, 2015) and their relatives (Kendler and Gardner, 1997; Yaralian *et al.*, 2000; Calkins *et al.*, 2004; Moreno-Izco *et al.*, 2015) present higher levels of schizotypy than in the general population. Moreover, it has also been

revealed that schizotypy co-occurs with schizophrenia in the same families (Kendler, Thacker and Walsh, 1996; Mata *et al.*, 2000; Appels *et al.*, 2004). In this regard, molecular studies have associated schizophrenia PRS with schizotypy, both in a sample of relatives of patients with psychotic disorders and a sample of healthy controls (van Os *et al.*, 2017), suggesting that overlapping aetiological factors might underlie both phenotypes (Fanous *et al.*, 2007). Accordingly, schizotypy has emerged as a useful framework for investigating aetiological factors of schizophrenia (Barrantes-Vidal, Grant and Kwapil, 2015).

2.2.2. Cognitive performance

Similarly to schizotypy, cognitive performance meets all the criteria to be considered an intermediate phenotype for psychotic disorders (Gur *et al.*, 2007; Swerdlow, Gur and Braff, 2015; Greenwood *et al.*, 2016; Mark and Toulopoulou, 2016). First, it is familial, with heritability estimates ranging from 20% to 80% (Greenwood *et al.*, 2007, 2016; Keshavan *et al.*, 2009; Keefe and Harvey, 2012; Bora *et al.*, 2014; Seidman *et al.*, 2015; Blokland *et al.*, 2017; Mollon *et al.*, 2018). Second, compared to healthy controls, patients with schizophrenia or bipolar disorder show a global cognitive impairment and a worse performance in specific dimensions, including working memory, attention/vigilance, verbal/visual learning and memory, executive functions (reasoning and problem solving), processing speed, social cognition and psychomotor control (Kurtz and Gerraty, 2009; Keefe and Harvey, 2012; Bourne *et al.*, 2013; Aas *et al.*, 2014; Szmulewicz *et al.*, 2015; Li *et al.*, 2020). Third, cognitive deficits are observed in individuals with psychosis before illness onset, are largely independent of clinical state and medication status, and seem to be stable over long term follow up (Rund *et al.*, 2015). Finally, they are also present in non-affected family members at higher rates than in the general population (Bora, Yucel and Pantelis, 2009a; Jabben *et al.*, 2010; Hu *et al.*, 2011; Papmeyer *et al.*, 2015; Schulze-Rauschenbach *et al.*, 2015; Bora, 2017).

Moreover, several studies have reported associations between schizophrenia-associated genes and cognitive performance, suggesting that the phenotypic correlation between cognition and liability to schizophrenia is explained by a shared

genetic variability (Fernandes *et al.*, 2013; Greenwood *et al.*, 2013, 2016; Ohi *et al.*, 2015; Trampush *et al.*, 2017; Zai *et al.*, 2017; Davies *et al.*, 2018; Toulopoulou *et al.*, 2018). In this sense, schizophrenia PRS has been associated with lower general cognitive performance (McIntosh *et al.*, 2013; Lencz *et al.*, 2014; Hubbard *et al.*, 2016; Nakahara *et al.*, 2018; Wang *et al.*, 2018) and alterations in particular cognitive domains, including speed of emotion, verbal reasoning, verbal-numerical reasoning, reaction time and memory (Germine *et al.*, 2016; Hagenaars *et al.*, 2016; Ranlund *et al.*, 2018). Also, recent studies have revealed the association between the PRS of bipolar disorder and different cognitive domains such as executive function or processing speed (Mistry *et al.*, 2019; Chang *et al.*, 2020).

2.3. Gene x Cannabis Interaction studies

There is compelling evidence that the use of cannabis increases the risk for psychosis (Minozzi *et al.*, 2010; Gage, Hickman and Zammit, 2016; Kelley *et al.*, 2016; Schoeler *et al.*, 2016; R.M. Murray *et al.*, 2017; Jones *et al.*, 2018; Mustonen *et al.*, 2018; Di Forti, Quattrone, Tom P Freeman, *et al.*, 2019; Patel *et al.*, 2020). Evidence also show that the association between the use of cannabis and the risk for psychosis follows a dose-response relationship (the OR of heaviest cannabis users compared with non-users is 3.90; Marconi *et al.*, 2016; see **Figure 15**) and that is dependent on the potency of cannabis (the OR of developing psychotic disorders increases to nearly five times when using high-potency cannabis; Di Forti *et al.*, 2019). Moreover, cannabis use has also been identified as a vulnerability factor for subclinical psychosis-related phenotypes, including psychotic experiences (Fergusson *et al.*, 2003; Henquet *et al.*, 2005; Wainberg *et al.*, 2021), schizotypy (Cohen *et al.*, 2011; Fridberg *et al.*, 2011; Schubart *et al.*, 2011; Szoke *et al.*, 2014) and brain function and structure alterations (Rabin, Zakzanis and George, 2011; Segev and Lev-Ran, 2012; Crane *et al.*, 2013; Sánchez-Torres *et al.*, 2013; Thames, Arbid and Sayegh, 2014; González-Pinto *et al.*, 2016; Núñez *et al.*, 2016; Castellanos-Ryan *et al.*, 2017; Nader and Sanchez, 2018; Duperrouzel *et al.*, 2019; Petker *et al.*, 2019).

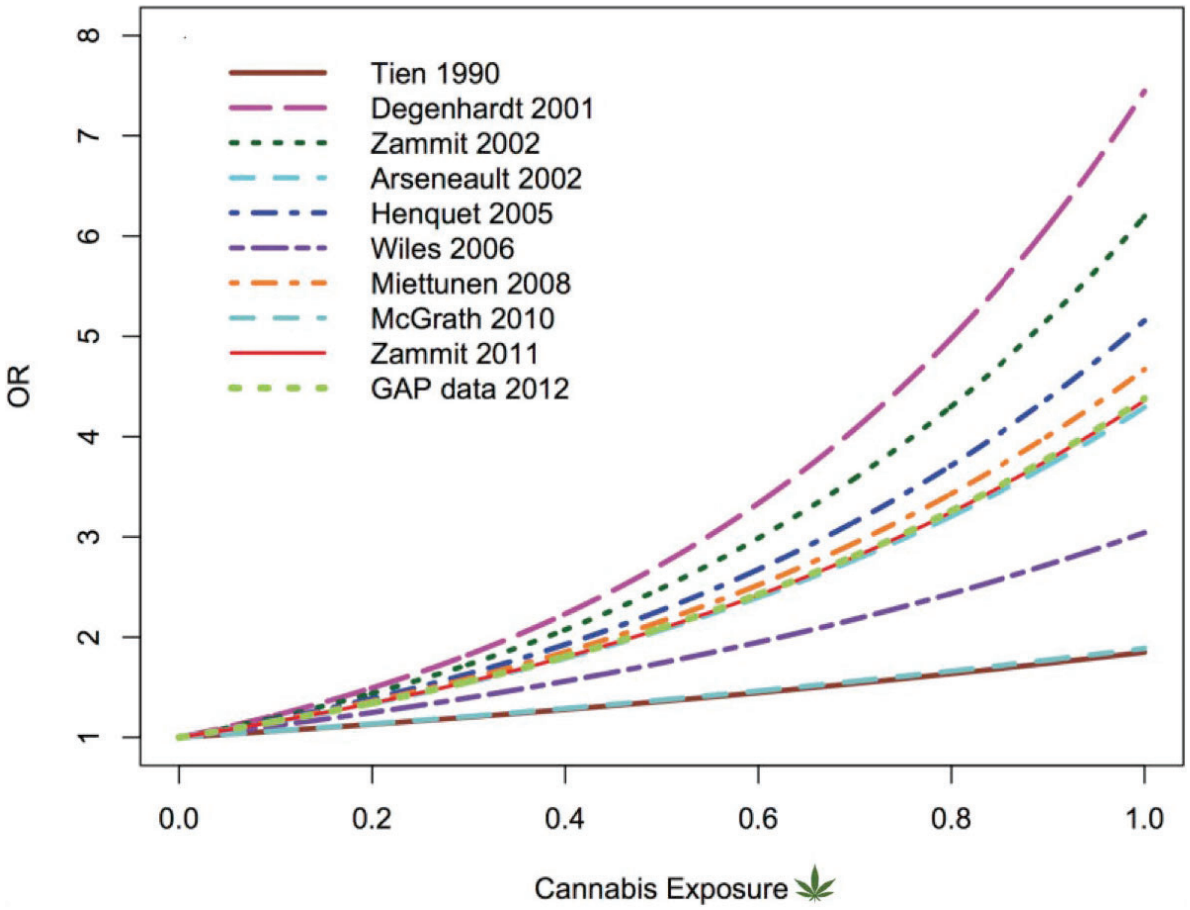


Figure 15. Estimated risk ratio (OR) of psychosis by the level of cannabis use in original studies. The x-axis shows the different levels of exposure on a scale of 0 to 1, whereas the y-axis shows the OR associated with each level. As observed in the graph, there is a consistent increase in the risk of psychosis-related outcomes with higher levels of cannabis exposure in all the included studies. Adapted from Marconi et al. (2016).

Although the use of cannabis increases the risk of psychosis, only a minority of individuals exposed to this factor become ill. Hence, genetic differences may render some individuals more vulnerable or resilient to the impact of cannabis use, suggesting that the effects of cannabis are modulated by an individuals' genetic background, resulting in a gene-environment interaction (GxE). Consistent with that, epidemiological studies have shown that individuals who are genetically vulnerable to psychosis show increased cannabis-induced psychotic symptoms (van Os *et al.*, 2002; Verdoux *et al.*, 2003; D'Souza *et al.*, 2005; Henquet *et al.*, 2005; Kahn *et al.*, 2011; Decoster *et al.*, 2012; Radhakrishnan, Wilkinson and D'Souza, 2014; van Winkel and GROUP Investigators, 2015; Wainberg *et al.*, 2021). Moreover, interaction studies have shown the interplay between particular schizophrenia-

associated genes (e.g. *COMT, AKT1*) and cannabis use on the risk for psychosis (Van Winkel *et al.*, 2011; Di Forti *et al.*, 2012; Radhakrishnan, Wilkinson and D'Souza, 2014) and on the expression of psychosis-associated intermediate phenotypes, such as cognitive performance or schizotypy levels (van Winkel *et al.*, 2011; Van Winkel *et al.*, 2011; Cosker *et al.*, 2018).

Accordingly, in order to shed some light on the understanding of the gene-environment interactions in the expression of schizophrenia psychopathology, the present thesis has investigated whether the relation between cannabis use and the presentation of two intermediate phenotypes associated with psychosis (schizotypy and cognitive performance) is modulated by genetic factors.

HYPOTHESES AND OBJECTIVES

The clinical heterogeneity of psychotic disorders is mirrored by a complex genetic architecture involving several types of genetic variants that interact with several environmental factors. According to this, a better understanding of these genetic and environmental influences may provide a way to dissect the biology of psychosis and, ultimately, allow improving the diagnosis and designs of new interventions and therapies. However, the study of the genetic basis of schizophrenia and other psychotic disorders, though, has a serious limitation in the high biological heterogeneity underlying the diagnosis itself. The heterogeneity of clinical profiles and the high phenotypic variability, in turn, causes uncertainty on the genetic results related to these disorders. Thus, the reduction of phenotypic complexity has become an essential step to contribute to the genetic dissection of brain complex phenotypes. In this thesis, such reduction has been proposed using different strategies, including:

a) The use of **family-based designs** (including families with at least one patient with a psychotic disorder). This strategy allows examining the degree of familial aggregation of a trait (also known as familiality). As explained above, the identification of a familial pattern for a trait (linked to the intermediate phenotype definition) is associated with a stronger phenotype–genotype correlation (Goldberg *et al.*, 2012).

b) The use of **intermediate phenotypes**; this is, quantifiable markers that might potentiate the identification of associated genetic factors (Gottesman and Gould, 2003). Intermediate phenotypes can be also studied in non-clinical populations in order to overcome the presence of multiple confounding factors inherent to psychosis.

c) The study of **gene-environment interactions**, which allows studying how the genotype-phenotype correlation is modulated by the effect of environmental factors.

General hypothesis

Accordingly, in line with the current approaches that try to disentangle the heterogeneity of psychotic disorders, we hypothesize that the knowledge of the biological mechanisms underlying these disorders will benefit from the use/identification of intermediate phenotypes and the study of their similarities/dissimilarities within/between families. Therefore, the detection of phenotypes that maximize the phenotype–genotype correlation is a first step in understanding the genetic underpinnings of psychotic disorders.

Also, taking into account molecular data highlighting the alteration of synaptic plasticity in the aetiology and pathophysiology of psychotic disorders, we hypothesize that genetic variability on genes involved in such mechanism and their modulation by an environmental risk factor such as cannabis use will explain part of the variance of the intermediate phenotypes.

Three specific hypotheses can be drawn from the above:

I. The study of the **familiality of intermediate phenotypes** of interest in psychotic disorders (i.e. schizotypy, cognitive performance and neurodevelopmental markers) will conduct to the identification of markers related with a higher genetic loading for these disorders (i.e. markers correlated with risk a genotypic background of risk).

   Individuals carrying or presenting these markers to a higher degree will represent a more genetically homogeneous group, which may facilitate the identification of specific genetic risk factors.

II. Genes involved in synaptic plasticity represent a candidate gene-set to be associated with psychotic disorders intermediate phenotypes. **Genetic variability** in these genes will be correlated with phenotypic variability in clinical and non-clinical samples.

III. **Gene-environment interactions** are involved in the variability of the severity of different intermediate phenotypes. Some of the detected genotype-phenotype correlations will be modulated by cannabis use in non-clinical samples.

Objectives

Concerning the former hypotheses, the present thesis has been focused on three different objectives:

I. To assess the **familiality** of clinical (schizotypy), cognitive (executive function, reasoning skills, attention, memory, working memory) and neurodevelopmental (dermatoglyphics) intermediate phenotypes of interest in families with at least one patient with an offspring diagnosed with a psychotic disorder, and to develop a methodology to estimate continuous score (**intrafamilial resemblance score, IRS**) that estimates the similarity of these traits among family members.

II. To study the correlates of **genetic variants** in genes encoding for different synapse function and regulation proteins (***DAOA*, *RGS4*, *AKT1, ZNF804A*)** with clinical (**schizotypy**), neurodevelopmental (**dermatoglyphics**) and **cognitive** intermediate phenotypes of interest in psychotic disorders.

In relation to the analysis of the genetic underpinnings of schizophrenia and the overlap with other disorders, we also aimed to study the role of both common and rare variants across two neurodevelopment disorders such as schizophrenia and autism. Accordingly, this aim included conducting a systematic review of the literature to analyse the involvement of particular genetic variants within genes encoding for scaffolding proteins across these psychiatric diagnoses.

III. To study the effect of **cannabis use** on the modulation of the genotype-phenotype correlations in **non-clinical samples**. Particularly, this aim included assessing whether cannabis use mediates the relationship between the *ZNF804A* gene and schizotypy, and the relationship between the *AKT1* gene and cognitive function.

SUPERVISOR'S REPORT ON IMPACT FACTOR

Supervisor's report on impact factor

The doctoral thesis "Towards disentangling the genetic complexity and clinical heterogeneity of psychotic disorders: from family-based approaches to gene-environment studies" is based on the original results obtained by Jordi Soler Garcia. These results are based on the analyses conducted in different family-based samples comprised of Spanish patients with psychotic disorders and their relatives and a sample of Spanish individuals from the general population.

Complementarily, this doctoral thesis also comprises a systematic review performed by Jordi Soler Garcia that aided the understanding of the genetic underpinnings of psychotic disorders. The review integrated the genetic and molecular studies conducted in the last decade to discuss the implications of the scaffolding proteins in the aetiology and pathophysiology of schizophrenia and autism spectrum disorders.

These results and the review have been published in the following International peer-reviewed journals:

1.

Soler J, Ferentinos P, Prats C, Miret S, Giralt M, Peralta V, Fañanás L, Fatjó-Vilas M

Familial Aggregation of Schizotypy in Schizophrenia-Spectrum Disorders and Its Relation to Clinical and Neurodevelopmental Characteristics

Journal of Psychiatric Research, 2017 Jan; 84:214-220
DOI: 10.1016/j.jpsychires.2016.09.026

This journal publishes peer-reviewed original research that contributes to the understanding of brain function and human behaviour. According to the Journal Citation Reports (Science Edition, 2016), the impact factor of the journal at the time of publication was 4.000, classified in the first quartile (Q1) of the area of psychiatry (ranking: 31/142).

2.

Soler J, Lera-Miguel S, Lázaro L, Calvo R, Ferentinos P, Fañanás L, Fatjó-Vilas M

Familial aggregation analysis of cognitive performance in early-onset bipolar disorder.

European Child and Adolescent Psychiatry, 2020 Dec; 29(12):1705-1716.
DOI: 10.1007/s00787-020-01486-8

This journal is the Europe's only peer-reviewed journal entirely devoted to child and adolescent psychiatry. It aims to further a broad understanding of psychopathology in children and adolescents. According to the Journal Citation Reports (Science Edition, 2019), the impact factor of the journal at the time of publication was 3.941, classified in the first decile (D1) of the area of pediatrics (ranking: 9/128).

3.

Soler J, Miret S, Lázaro L, Parellada M, Martín M, Lera-Miguel S, Rosa A, de Castro-Catala M, Cuesta M, Fañanás L, Krebs MO, Fatjó-Vilas M

Influence of DAOA and RGS4 genes on the risk for psychotic disorders and their associated executive dysfunctions: A family-based study.

European Psychiatry, 2016 Feb; 32:42-7.
DOI: 10.1016/j.eurpsy.2015.11.002

This journal, which is the official journal of the European Psychiatric Association, publishes the latest advances in the field of psychiatry, including new developments in diagnosis and treatment and advances in the biological underpinnings of mental, behavioural and cognitive function in clinical and general population samples. According to the Journal Citation Reports (Science Edition, 2015), the impact factor of the journal at the time of publication was 3.912, classified in the first quartile (Q1) of the area of psychiatry (ranking: 32/142).

4.

Soler J, L Fañanás,M Parellada, MO Krebs, GA Rouleau, M Fatjó-Vilas

Genetic variability in Scaffolding Proteins and the risk for schizophrenia and autism spectrum disorders: a systematic review.

Journal of Psychiatry & Neuroscience, 2018 May 28; 43:223–244.
DOI: 10.1503/jpn.170066

This journal publishes peer-review original research and review articles at the intersection of psychiatry and neuroscience that advance our understanding of the neural mechanisms involved in the aetiology and treatment of psychiatric disorders. According to the Journal Citation Reports (Science Edition, 2017), the impact factor of the journal at the time of publication was 5.365, classified in the first quartile (Q1) of the area of psychiatry (ranking: 17/140).

5.

Soler J, Arias B, Moya J, Ibañez MI, Ortet G, Fañanás L, Fatjó-Vilas M

ZNF804A gene and cannabis use: interaction on the risk for psychosis in a non-clinical sample.

Progress in Neuropsychopharmacology & Biological Psychiatry, 2019 Mar 8; 9:174-180.
DOI: 10.1016/j.pnpbp.2018.08.009

This multidisciplinary journal publishes studies dealing with experimental and clinical aspects of neuropsychopharmacology and biological psychiatry. According to the Journal Citation Reports (Science Edition, 2018), the impact factor of the journal at the time of publication was 4.315, classified in the first quartile (Q1) of the area of psychiatry (ranking: 28/146).

6.

M Fatjó-Vilas*, **J Soler***, M I Ibáñez, J Moya-Higueras, G Ortet, M Guardiola-Ripoll, L Fañanás, B Arias

* *Joint first authorship*

Analysis of AKT1 and cannabis moderation effects on cognitive performance in healthy subjects.

Journal of Psychopharmacology, 2020 Sep; 34(9):990-998
DOI: 10.1177/0269881120928179

This journal publishes peer-reviewed original research and review articles on preclinical and clinical aspects of psychopharmacology. The journal provides an essential forum for researchers and clinicians on the effects of drugs on animal and human behaviour and the mechanisms underlying these effects. According to the Journal Citation Reports (Science Edition, 2019), the impact factor of the journal at the time of publication was 3.121, classified in the second quartile of the area of psychiatry (ranking: 57/155).

Accordingly, we confirm the quality of the published articles.

Signed by Dr Mar Fatjó-Vilas and Dr Lourdes Fañanás

Barcelona, May 14th 2021

PUBLICATIONS

1.

Familial Aggregation of Schizotypy in Schizophrenia-Spectrum Disorders and Its Relation to Clinical and Neurodevelopmental Characteristics.

Soler J, Ferentinos P, Prats C, Miret S, Giralt M, Peralta V, Fañanás L, Fatjó-Vilas M

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Familial aggregation of schizotypy in schizophrenia-spectrum disorders and its relation to clinical and neurodevelopmental characteristics

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ABSTRACT

Introduction: This study explored schizotypy as a familial liability marker for schizophrenia-spectrum disorders (SSD) by examining: 1) the aggregation of schizotypy in families with a SSD patient, 2) whether familial resemblance of schizotypy is associated with ridge dissociations (RD), another SSD liability marker, 3) whether schizotypy aggregation patterns influence patients' psychopathology.
Methods: The sample comprised 30 SSD patients and 82 healthy first-degree relatives. Schizotypy was assessed using the Structured Interview for Schizotypy-Revised (SIS-R). Patients' psychopathology was evaluated using the Comprehensive Assessment of Symptoms and History (CASH). RD were identified as anomalies of the dermal ridge junction. Familiality of SIS-R was investigated using a linear mixed model (LMM) and its strength was assessed using an intraclass correlation coefficient (ICC). Another LMM using the absolute differences in SIS-R scores between all possible pairs of relatives as the dependent variable was fitted to obtain an intra-family resemblance score, a family-specific indicator of resemblance of SIS-R scores within each family.
Results: 1) Schizotypy was familial (ICC = 0.30); families with high resemblance displayed low schizotypy, whereas families with low resemblance included at least one healthy relative with high schizotypy ($p < 0.001$). 2) Relatives with RD had higher SIS-R scores ($p = 0.018$) and belonged to families with discordant schizotypy scores among members ($p < 0.001$). 3) Patients from high schizotypy families showed more severe disorganized symptoms at the psychotic episode ($p = 0.035$) and 1 year later ($p = 0.011$).
Conclusions: Schizotypy is a marker of vulnerability for SSD that runs within a subgroup of families. The schizotypy familial aggregation pattern correlates with RD in relatives and with patients' psychopathology.

1. Introduction

Schizotypy is a set of personality traits encompassing behaviours, cognitions and emotions that resemble the signs and symptoms of schizophrenia in the general population. Schizotypy encompasses perceptual impairments and unusual views or ideas, a loss of normal emotional, physical and social functions and odd

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behaviour and speech, among other traits (Raine, 2006). Due to the clinical resemblance between schizotypy and schizophrenia it has been suggested that overlapping aetiological factors might underlie the two phenotypes (Fanous et al., 2007). Therefore its study constitutes a useful framework within which to investigate aetiological factors of schizophrenia spectrum disorders (SSD) (Barrantes-Vidal et al., 2015).

The dimensional approach of schizotypy regards it as a personality trait that is continuously distributed in the population (Claridge, 1997), which ranges from low schizotypy and psychological health to extremely high schizotypy and potential dysfunction in the form of psychosis (Nelson et al., 2013). Therefore, this model assumes that schizotypal symptoms in the healthy population are similar but quantitatively milder than those observed in schizophrenia patients. In this regard, schizotypy has been found to be elevated among SSD patients (Brosey and Woodward, 2015; Chapman et al., 1978) and their relatives (Calkins et al., 2004; Kendler and Gardner, 1997; Moreno-Izco et al., 2015; Yaralian et al., 2000). Moreover, it has been reported that both schizotypy and schizophrenia co-occur in the same families (Kendler et al., 1996; Mata et al., 2000) and that healthy parents of patients with schizophrenia that had a family history of SSD displayed more schizotypal traits than parents without familial antecedents (Appels et al., 2004). In addition, a recent study has shown correlations between schizotypy, cognitive impairments and psychosocial functioning, both in psychotic and healthy individuals (Brosey and Woodward, 2015). Also, the same authors have reported a modest association of schizotypy with the negative and general psychopathology scores (PANSS). Accordingly, from studies in which patients and/or relatives are compared with controls, there is support for a familial association between schizotypy and schizophrenia, and therefore, schizotypy is considered a clinical marker of latent liability for schizophrenia.

Another avenue to the analysis of this familial association is to examine the familial aggregation of schizotypy (also known as familiality) in families with an individual with schizophrenia. This kind of approach aims to detect phenotypes that maximize the phenotype–genotype correlation as a first step in disentangling the molecular genetic underpinnings of disorders. As an example, this strategy has been used to dissect the heterogeneity of major depressive disorder (Ferentinos et al., 2015), where age at onset was reported to significantly aggregate in families at a moderate degree. As regards to schizotypy, some studies have reported evidence on familiality of the schizotypy dimension anhedonia by analyzing the correlation for schizotypy between pairs of first-degree relatives (patient-sibling, patient-parent or college student-parent) (Berenbaum and McGrew, 1993; Clementz et al., 1991; Grove et al., 1991).

Besides estimating the global degree of familiality of a phenotype in a sample of pairs of related individuals, it is also possible to calculate similarity among all family members on that trait for each family of the sample (here called the intra-family resemblance score, IRS). This approach might allow the classification of a set of families according to their members' resemblance on a phenotype. Therefore, the IRS can be used to differentiate families in which members share similar scores from those families in which members show discordant scores. We propose that the investigation of factors underlying IRS differences among families might represent a valuable strategy for defining aetiological subgroups.

Based on the altered neurodevelopment hypothesis of schizophrenia (Rapoport et al., 2012), research has shown that neurodevelopmental markers typically linked to schizophrenia, such as neurological soft signs or minor physical anomalies, are also associated with schizotypy (Barrantes-Vidal et al., 2003; Gourion et al., 2004; Hans et al., 2009; Solanki et al., 2012). In this regard, the dermatoglyphic pattern, which is the epidermal ridge pattern that forms prints on the fingers, hands and soles, has also been previously associated with schizotypy in healthy relatives and controls (Chok and Kwapil, 2005; Gabalda and Compton, 2010; Rosa et al., 2000b). Deviations and aberrations in dermatoglyphic patterns are considered potential aetiopathogenic markers of schizophrenia risk as they reflect disruptions of gestational ectodermal development (Golembo-Smith et al., 2012).

Dermatoglyphic measures typically rely on quantitative traits such as ridge counts. In this sense, it has been shown that patients with schizophrenia display an increased prevalence of reduced ridge counts (total finger ridge count and a–b ridge count) (Bramon et al., 2005; Fañanas et al., 1996; Fañanás et al., 1990; Fearon et al., 2001; Jelovac et al., 1999). Qualitative traits that reflect the abnormal configuration of ectodermic derivates during the prenatal period have been less well studied in schizophrenia. Ridge dissociations (RD) are defined as short broken segments of lines that cover the areas with dermatoglyphic patterns in a disorganized way (Cummins and Midlo norms, 1943). Although RD have been reported to be more frequent in SSD patients (Rosa et al., 2002, 2000a) and their first-degree relatives (Fatjó-Vilas et al., 2008) compared to healthy subjects, to the best of our knowledge there have been no studies assessing the relationship between schizotypy and RD in healthy relatives of schizophrenia patients.

In line with the above, we aimed to explore the role of schizotypy as a familial liability marker for SSD. Therefore, we first examined the aggregation of schizotypy in families including a patient with SSD. Based on the detected familiality of schizotypy, we then estimated the Intrafamily Resemblance Score (IRS), which allowed the classification of families according to the level of schizotypy similarity between healthy members. Second, we investigated whether the schizotypy familial pattern is related to the presence of another marker of schizophrenia liability such as RD. Finally, we explored whether these schizotypy familial patterns influence patients' psychopathological symptoms.

2. Methods

2.1. Sample

The sample comprised 30 patients with SSD and their 82 healthy first-degree relatives (23 fathers, 29 mothers and 30 siblings) (Table 1). Patients' DSM-IV-TR diagnoses included: schizophrenia (n = 16), schizophreniform disorder (n = 7), psychotic disorder not otherwise specified (n = 6) and schizoaffective disorder (n = 1). After 1 year all diagnoses remained stable except for one patient with a psychotic disorder not otherwise specified that was re-diagnosed with schizophrenia.

Patients' exclusion criteria included any major medical illnesses that could affect brain function, neurological conditions, history of head trauma with loss of consciousness and drug abuse/dependence. Relatives' exclusion criteria included any major psychiatric disorder (SSD, Bipolar Disorder and Major Depression Disorder), any major medical illnesses that could affect brain function, neurological conditions and history of head trauma with loss of consciousness. All individuals underwent a clinical interview in order to evaluate their present and lifetime history of mental illness and/or treatment with psychotropic medication.

All participants provided written consent after being informed of the study procedures and implications. The study was performed in accordance with the guidelines of the institutions involved and

approved by the local ethics committee of each participating centre.

2.2. Phenotype assessment

Both patients and relatives underwent schizotypy assessment, which was carried out by an experienced psychiatrist by means of the Structured Interview for Schizotypy-Revised (SIS-R) (Vollema and Ormel, 2000). SIS-R is an interview instrument that measures a broad range of schizotypal symptoms and signs by applying standardized rating and scoring procedures. The SIS-R total score was used in this study.

Patients' age at onset of first psychotic symptoms was assessed with the Symptom Onset in Schizophrenia (SOS) inventory (Perkins et al., 2000) and psychopathological symptoms by means of the Comprehensive Assessment of Symptoms and History (CASH) (Andreasen et al., 1992). According to CASH, symptoms are grouped in six dimensions: psychotic, negative, depression, disorganization, mania and catatonia. Patients were evaluated at two different times: during the psychotic episode and 1 year later (mean time between evaluations (sd) = 1.00 (0.85) year).

Bilateral fingers and hand prints were obtained from all participants using non-inky specific methods (Prints-kit, Printscan Verification Systems Ltd). Ridge dissociations (RD) were identified as clear anomalies of the dermal ridge junction according to Cummins and Midlo norms (1943). RD evaluation was available for 57 individuals, as poor quality prints were excluded.

2.3. Statistical modelling

All statistical analyses were implemented with Stata v. 14 (StataCorp, 2013).

2.3.1. Schizotypy familiality

Familiality of SIS-R was investigated separately both in the total sample and in healthy relatives only (excluding patients), in order to avoid the confounding effect of the disorder itself. Since SIS-R had a positively skewed distribution, a square root (sqrt) transformation was first applied. Familiality was analyzed by means of a two-level linear mixed model (LMM) with the transformed SIS-R total score as the dependent variable, patient status (only in the total sample analysis), sex and age as fixed effect covariates and family as random effect (subjects nested within families). The familiality of SIS-R was documented if the variance of the random effect of family was significantly greater than zero. Then, the strength of the familial effect was measured by calculating the family-level residual intraclass correlation coefficient (ICC). The ICC score ranges from 0 to 1, with 0 suggesting no aggregation and 1 complete familial aggregation for the trait.

2.3.2. Estimation of the Intrafamily Resemblance Score (IRS)

In the next step, we developed the methodology to calculate the *Intrafamily Resemblance Score (IRS)*. First, we created a new dataset comprising all possible pairs of healthy family members within families, therefore including N*(N-1)/2 pairs for each family of N healthy members. We then fitted a two-level LMM with absolute differences in SIS-R total scores (sqrt transformed) between the members of each pair as the dependent variable, sex and age of both pair members as fixed effect covariates and family as random effect (subjects nested within families). Since each family comprised only N-1 independent pairs, a family size weight of 2/N was applied in this model (Suarez and Van Eerdewegh, 1984). We finally calculated random effect estimates (best linear unbiased predictions, BLUPs) for each family to obtain the IRS score, a continuous parameter characteristic of each family that indicated the degree of intra-family similarity in SIS-R total scores after adjusting for the effect of covariates. The IRS score is, by definition, normally distributed with a mean of 0. Lastly, IRS scores were multiplied by −1 to facilitate interpretation: higher IRS scores indicate higher resemblance among family members, whereas lower IRS scores indicate lower resemblance (Fig. 1).

2.3.3. Schizotypy familiality and the presence of ridge dissociations (RD)

A linear regression of IRS scores on presence of RD (adjusting for sex and age) was used to assess whether schizotypy familial resemblance in healthy relatives predicted the presence of these dermatoglyphic anomalies in patients. A linear regression of SIS-R total scores on presence of RD (adjusting for sex and age) was also fitted in patients to assess whether those with RD had higher schizotypy scores than those without RD.

Separately, similar regressions (of IRS scores and SIS-R total scores on presence of RD after adjusting for sex and age) were fitted in healthy relatives using robust standard errors to account for intrafamily correlations.

2.3.4. Schizotypy familiality and patients' clinical characteristics

Linear regressions of IRS scores on CASH dimensions (adjusting for sex and age) were used to assess whether schizotypy familial resemblance is associated with patients' CASH scores.

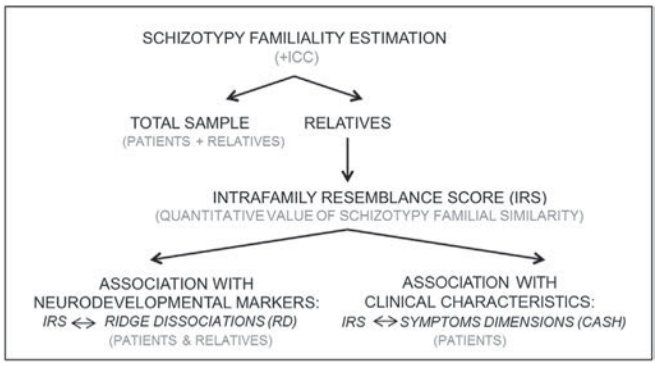

Fig. 1. Flowchart with the different analyses of the study. Firstly, familiality (ICC) of SIS-R was assessed both in the total sample and only in relatives. After ranking families according to the IRS, this measure was used to analyze whether schizotypy familial resemblance is associated with neurodevelopmental characteristics (RD) in patients and relatives and clinical characteristics (CASH) of patients.

3. Results

3.1. Sample characteristics

The sample characteristics are described in Tables 1 and 2. Patients' mean age at onset was 21.86 (SD = 3.50) and the mean duration of illness was 21.98 months (SD = 21.32). The group (patients, fathers, mothers and siblings) had a significant effect on SIS-R: $F = 14.55$ $p < 0.001$. Post-hoc analyses showed that differences were found between patients and each of the three groups of relatives ($p < 0.001$).

3.2. Schizotypy familiality and estimation of the Intrafamily Resemblance Score (IRS)

In the LMM to assess the familiality of SIS-R, the variance of the

Table 1
Characteristics of the sample. (SD: Standard Deviation, SIS-R: Structured Interview for Schizotypy-Revised).

	Gender (% males)	Mean age at interview (SD)	Mean SIS-R score (SD)	Presence of ridge dissociations (% of individuals)
Patients n = 30	70%	23.89 (3.17)	19.51 (8.82)	31%
Mothers n = 29	0%	52.13 (6.00)	7.07 (5.97)	12%
Fathers n = 23	100%	53.99 (5.33)	9.77 (10)	28%
Siblings n = 30	43.30%	27.19 (5.10)	8.12 (5.57)	19%

Table 2
Comprehensive Assessment of Symptoms and History (CASH) dimension scores of patients (Mean (Standard Deviation)).

	Episode	1 year follow-up
Psychotic	2.36 (0.98)	0.51 (0.67)
Negative	2.62 (0.77)	1.91 (0.71)
Depression	2.4 (0.72)	1.46 (0.69)
Disorganization	1.92 (0.95)	0.61 (0.86)
Mania	1.20 (1.0)	0.25 (0.51)
Catatonia	1.20 (1.29)	0.03 (0.18)

family random effect was significantly greater than zero, both in the total sample ($p < 0.001$) and in the subset of healthy relatives ($p = 0.034$). Schizotypy showed a moderate degree of familiality: the ICC (95% CI) for SIS-R was 0.30 (0.13–0.56) and 0.24 (0.06–0.59), respectively.

Afterwards, the IRS was calculated in relatives and families were ranked accordingly (i.e. ordered according to the healthy relatives resemblance). IRS scores were negatively associated with the mean SIS-R score of the relatives of the family ($\beta = -0.10$ SE $= 0.02$ $p < 0.001$ $R^2 = 0.38$); meaning that families with higher resemblance included relatives all with similarly low schizotypy scores, whereas families with lower resemblance were those with heterogeneous scores on SIS-R among relatives (Fig. 2). Then, these latter families are defined by discordant schizotypy scores among their members (with at least one relative with high schizotypy score) and hereinafter they will be referred as discordant families.

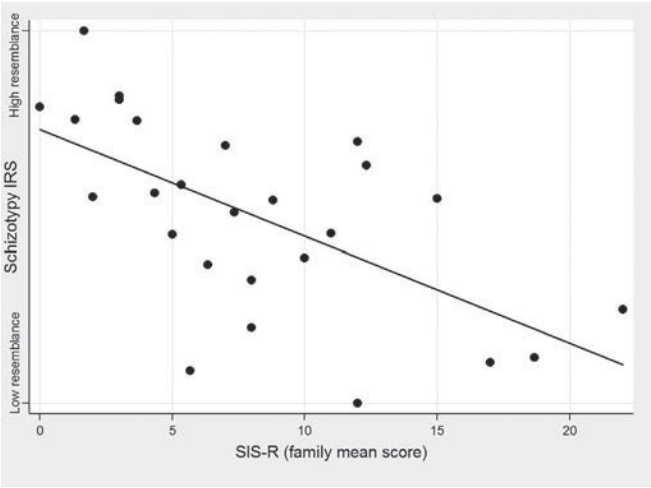

Fig. 2. Graph showing the relationship between schizotypy Intrafamily Resemblance Score (IRS) and mean SIS-R score of each family. Each dot represents one family. Schizotypy IRS was negatively associated with the mean SIS-R score of the family ($\beta = -0.10$ $p < 0.001$).

3.3. Schizotypy familiality and the presence of ridge dissociations (RD)

We investigated whether the relatives' schizotypy resemblance pattern was related to the presence of RD. In this regard, relatives with RD ($n = 12$) showed lower IRS than those without ($n = 48$) ($\beta = -1.09$ SE $= 0.29$ $p < 0.001$ $R^2 = 0.26$); meaning that individuals with dermatoglyphic anomalies belonged to discordant families for schizotypy. Furthermore, relatives with RD presented higher SIS-R scores than those without: (13.27 (10) vs 6.5 (6.10), respectively; $\beta = 0.93$, SE $= 0.36$, $p = 0.018$, $R^2 = 0.15$). Patients with and without RD did not differ in their IRS scores and SIS-R total scores.

3.4. Schizotypy familiality and patients' clinical characteristics

We explored whether there was a relationship between the relatives' schizotypy resemblance pattern and the patients' psychopathological profile. We found that IRS scores were negatively correlated with disorganized symptomatology both at the psychotic episode ($\beta = -0.45$ SE $= 0.20$ $p = 0.035$ $R^2 = 0.21$) and at the 1-year follow-up ($\beta = -0.49$ SE $= 0.17$ $p = 0.011$ $R^2 = 0.28$). These results indicate that patients belonging to discordant families for schizotypy tend to present more severe disorganized symptoms (Fig. 3). There was no significant association with any other CASH dimensions.

4. Discussion

This study examined the familial aggregation of schizotypy in

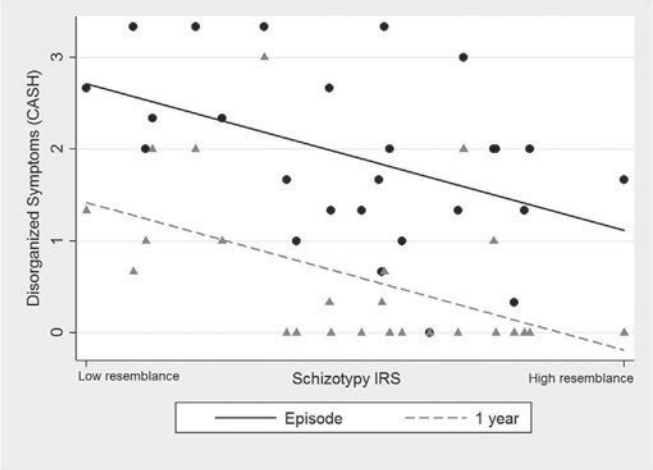

Fig. 3. Scatterplot of the regression of schizotypy Intrafamily Resemblance Score (IRS) and disorganized symptoms (CASH). Patients from families with a higher genetic loading tended to present more severe disorganized symptoms both at the episode (dots and solid line) and at the 1-year follow-up (triangles and dashed line).

families including a patient with SSD (first aim) and related it to neurodevelopmental characteristics of both patients with SSD and their relatives (second aim) as well as the patients' clinical profile (third aim). To accomplish the second and third aims, we extended the analysis of familiality *per se* by estimating the Intrafamily Resemblance Score (IRS), which is a continuous family-specific parameter of similarity between individuals of each family. The analysis of the familiality of a trait (phenotypic resemblance among family members) is assumed to be a useful strategy to guide the stratification of families in order to reduce heterogeneity and to

facilitate the identification of genetically more homogeneous forms of psychotic disorders (Peralta et al., 2015). In addition, implementation of the IRS method in our sample enabled us to adapt the approach of familiality in order to focus on the family as the element of study and to differentiate those families in which relatives had similar schizotypy levels from families in which relatives were discordant. To this respect, this approach adds on previous studies based on groups comparison (patients *vs* controls or relatives *vs* controls) (e.g. Brosey and Woodward, 2015; Moreno-Izco et al., 2015) or on familial aggregation based on the analysis of correlation between pairs of family members (Berenbaum and McGrew, 1993; Clementz et al., 1991; Grove et al., 1991). Therefore, our results are complementary to previous ones but cannot be directly compared to them.

According to our first aim, we found that schizotypy was familial in families including a patient with SSD both in the total sample (ICC = 0.30) and in the subset of relatives (ICC = 0.24). This is in line with previous studies that have reported that schizotypy is genetically influenced, with a heritability at approximately 50% (Linney et al., 2003). Afterwards, we calculated the IRS score in relatives and then classified families accordingly. We found that similarity among family members is due to low schizotypy scores of the first degree relatives, whereas families with low resemblance included at least one healthy relative who presented a discordant high score on schizotypy. This result converges partially with those studies that have shown the schizotypy correlation between patients with schizophrenia and a first degree relative (Clementz et al., 1991; Grove et al., 1991). However, also based on these studies of schizotypy familial aggregation and on evidence on non-random mating in psychiatric populations (Nordsletten et al., 2016), we would have expected to also find families concordant for high schizotypy scores. Due to the absence of previous studies on intrafamilial resemblance of schizotypy, further studies in larger samples are needed to evaluate and discuss whether the lack of concordant families for high schizotypy is related to the limited sample size of our study.

Nevertheless, the detected variability in concordance-discordance for schizotypy between families is also highly informative in terms of analyzing the role of schizotypy as a familial liability marker. In this regard, discordant families could be conceptualized as a subgroup that express or carry vulnerability for SSD that is mediated by common factors with schizotypy. This result adds evidence to the role of schizotypy as an endophenotypic marker (Grant, 2015), which would indicate the liability background of certain individuals/families to develop SSD.

Having considered schizotypy as a vulnerability marker of SSD that runs within a subgroup of families, we analyzed whether schizotypy familial aggregation is associated with the presence of another SSD vulnerability marker such as RD. We found that relatives with RD had higher schizotypy scores and belonged to discordant families. This is consistent with previous studies in which dermatoglyphic alterations were associated with schizotypy in healthy individuals (Chok and Kwapil, 2005; Chok et al., 2005; Rosa et al., 2000b). Thus, these results provide evidence that both schizotypy and RD could be, at least partially, influenced by some shared risk factors related to neurodevelopmental processes, which in turn could increase the susceptibility to SSD.

Finally, we found that the schizotypy aggregation pattern in relatives might be predictive of some patients' clinical characteristics. According to our results, higher mean familial schizotypy scores are associated with more severe disorganized symptoms in patients. On the one hand, this association is consistent with previous studies that have reported a high heritability of disorganization and related symptoms ($h^2 \cong 0.65$) (McGrath et al., 2009; Peralta et al., 2015) and that have also shown the familial aggregation of this dimension (Cardno, 2001; Loftus et al., 1998; Rietkerk et al., 2008; Vassos et al., 2008). On the other hand, our results are also in line with data on the association of disorganization with an increased risk for psychosis in relatives (Cardno, 2001; McGrath et al., 2004) and on the relationship of schizotypy dimensions with cognitive disorganization (Linney et al., 2003).

Some limitations of this study must be acknowledged. First, the moderate sample size and the lack of a replication sample are the main limitations of this study. However, since the estimation of Intrafamily Resemblance Score (IRS) was only performed with healthy relatives, the sample size was a compromise in exchange for avoiding the confounder of the disorder itself. Second, in order to explore the familiality of schizotypy more thoroughly, it would be interesting to use some other evaluation scale that includes the assessment of schizotypy dimensions. To this respect, previous studies have shown evidence of a stronger familial resemblance for anhedonia (Berenbaum and McGrew, 1993; Clementz et al., 1991; Grove et al., 1991) and have also suggested that the dimensional components of schizotypy are relatively genetically independent (Linney et al., 2003). Third, we calculated the IRS of each family by taking into account genetically related pairs (parents and offspring) and genetically non-related pairs (fathers and mothers). Due to the evidence for non-random mating in schizophrenia (Nordsletten et al., 2016; Parnas, 1988), it would be interesting to calculate the IRS of each family considering only the SIS-R scores of the genetically related pairs in a larger sample. In order to overcome this issue we performed some sensitivity analyses. We estimated the IRS for SIS-R excluding the parental pairs who were not genetically related. A LMM was performed in families with at least two genetically related pairs (father-sibling, mother-sibling or sibling-sibling, n = 15). The fitted LMM was significant and the two estimates of IRS (including and excluding genetically unrelated pairs of relatives) were strongly correlated ($r^2 = 0.95$ $p < 0.0001$). These results indicate the validity of the IRS score including parental pairs. Fourth, although healthy relatives of the sample displayed significantly lower levels of schizotypy than patients, the lack of a control group does not allow to test whether relatives show intermediate scores between patients and controls (as observed in other studies) (Shah et al., 2015; Solanki et al., 2012). Fifth, analyses on patients' CASH clinical dimensions might warrant the inclusion of neuroleptic treatment dosage as a covariate. Although all patients were treated with atypical antipsychotics, more specific treatment information was not available for the current sample. Finally, if multiple testing were addressed in the overall analyses not all the findings would remain significant. Thus, although the results cannot be dismissed completely, since they are consistent with previous studies, they should be interpreted with caution and replication studies are needed.

In conclusion, our results support the notion that the investigation of factors underlying schizotypy familiality might represent a valuable strategy to potentially define aetiological subgroups.

Conflicts of interest

There are no conflicts of interest.

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Contributors

LF, JS, SM and MFV designed the study. JS, PF, CP and MFV undertook the statistical analysis. JS and MFV wrote the first draft of the manuscript. All authors advised on interpretation of the results and contributed, read and approved the final manuscript.

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Supervisor's report on the contribution of the PhD applicant to the article

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Dr Lourdes Fañanás, professor at the Department of Evolutionary Biology, Ecology and Environmental Sciences of the Faculty of Biology, University of Barcelona,
as the supervisors of the present doctoral thesis by Jordi Soler Garcia, hereby certify that the participation of the PhD applicant in the article *"Familial Aggregation of Schizotypy in Schizophrenia-Spectrum Disorders and Its Relation to Clinical and Neurodevelopmental Traits"* included the following tasks:

- Conception and design of the study
- Statistical analyses
- Writing of the first manuscript draft
- Critical revision of the article for intellectual content

Dr Mar Fatjó-Vilas and Dr Lourdes Fañanás

Barcelona, May 14th 2021

2.

Familial aggregation analysis of cognitive performance in early-onset bipolar disorder.

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ORIGINAL CONTRIBUTION

Familial aggregation analysis of cognitive performance in early-onset bipolar disorder

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Abstract

We analysed the familial aggregation (familiality) of cognitive dimensions and explored their role as liability markers for early-onset bipolar disorder (EOBD). The sample comprised 99 subjects from 26 families, each with an offspring diagnosed with EOBD. Four cognitive dimensions were assessed: reasoning skills; attention and working memory; memory; and executive functions. Their familiality was investigated in the total sample and in a subset of healthy relatives. The intra-family resemblance score (IRS), a family-based index of the similarity of cognitive performance among family members, was calculated. Familiality was detected for the attention and working memory (AW) dimension in the total sample (ICC = 0.37, $p = 0.0004$) and in the subsample of healthy relatives (ICC = 0.37, $p = 0.016$). The IRS reflected that there are families with similar AW mean scores (either high or low) and families with heterogeneous scores. Families with the most common background for the AW dimension (IRS > 0) were selected and dichotomized in two groups according to the mean family AW score. This allowed differentiating families whose members had similar high scores than those with similar low scores: both patients ($t = -4.82$, $p = 0.0005$) and relatives ($t = -5.04$, $p < 0.0001$) of the two groups differed in their AW scores. AW dimension showed familial aggregation, suggesting its putative role as a familial vulnerability marker for EOBD. The IRS estimation allowed the identification of families with homogeneous scores for this dimension. This represents a first step towards the investigation of the underlying mechanisms of AW dimension and the identification of etiological subgroups.

Keywords Early-onset bipolar disorder · Cognitive performance · Attention · Working memory · Familial aggregation

Introduction

Bipolar disorder (BD) is a severe psychiatric disorder with a worldwide prevalence of around 2.4% [1]. BD is marked by clinical and pathophysiological heterogeneity. In addition to the characteristic mania and depression episodes

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of BD [2], cognitive dysfunction is also a core symptom that has been the subject of intensive research over the past decade because of its negative impact on socio-occupational outcome, quality of life and prognosis [3–5].

Despite the fact that the genetic component of BD is well established (heritability is estimated to be about 58–93% [6, 7]), its molecular underpinnings are poorly understood [8]. Such an understanding would lead to the identification of phenotypic subgroups through analysis of the disorder's clinical and pathophysiological heterogeneity.

Early age at onset has traditionally been considered to be associated with a more homogeneous aetiological background [9–11]. The term early-onset bipolar disorder (EOBD) is usually used to define cases that begin during childhood and adolescence; however, there is not consensus on a specific age cut-off [12–14].

As a number of studies have reported, EOBD is a subgroup with greater genetic/familial vulnerability [15–17]. For instance, Preisig et al. (2016) described that the offspring of parents with EOBD had a 7.9-fold increased risk of BD than those with parents with adult-onset BD [18].

EOBD has also been associated with greater severity, poorer prognosis and higher rates of comorbidity [19–21]. In this regard, the poorer functional outcome found in EOBD compared to adult-onset BD suggests worse cognitive performance among the youngest patients [22]. However, studies on EOBD have shown a non-unitary variety of cognitive deficits [23–28]. In this line, a recent systematic review and meta-analysis concluded that euthymic youths (aged ≤ 18 years) with BD exhibited significant cognitive dysfunction encompassing verbal and visual learning and memory and working memory, compared to healthy controls [29].

Analysis of cognitive heterogeneity in BD has been based primarily on two approaches. First, previous studies have identified different subgroups of patients with EOBD based on the differences in cognitive performance among patients [30–37]. Second, analyses based on unaffected first-degree relatives of patients with BD (obligate carriers of a certain degree of genetic risk) have found that they underperform compared to healthy subjects in different cognitive dimensions [38–43], and have indicated that verbal learning, processing speed, visual memory and working memory performance are promising intermediate phenotypes of familial risk for BD [44–48].

In addition, the hypothetical role of cognition as a marker of BD genetic loading has also been supported by studies that have included subjects at clinical and/or genetic risk. Olvet et al. found that global cognition is significantly impaired in at-risk subjects who later developed BD [49], while Ratheesh et al. (2013) reported deficits in processing speed, executive functions and general intellectual abilities in at-risk subjects who developed BD at follow-up as compared to healthy controls [50].

Taken together, these data suggest that there is great heterogeneity in the cognitive performance of BD patients, relatives and genetically at-risk individuals of BD, and pave the way for the identification of cognitive profiles, not only at individual level but also at family level. To this respect, the pattern of aggregation in families remains unexplored.

Therefore, the analysis of the familiality of a trait (phenotypic resemblance among family members) emerges as an approach to guide the stratification of families and facilitate the identification of liability markers that can potentially help to identify genetically more homogeneous forms of a disorder which could benefit of specific treatments or intervention strategies.

In addition to estimating the global degree of the familiality of a phenotype in a sample, it is also possible to quantitatively estimate the similarity of a trait among family members (herein referred to as the intra-family resemblance score, IRS). The IRS can, therefore, be used to differentiate families in which members have similar scores from those in which members show discordant scores. As we have already reported, the investigation of factors underlying IRS differences among families might represent a strategy for defining aetiological subgroups [51]. Accordingly, in the current study, we characterized the familial aggregation of four cognitive dimensions (reasoning skills; attention and working memory; memory; and executive functions) with a view to exploring the role of these dimensions as familial liability markers for EOBD. These analyses were conducted in families with an offspring diagnosed with a BD and in a subsample including only the relatives with no psychiatric lifetime diagnoses. This double approach allowed considering the possible confounding effects of disease status and medication in the complete sample as compared to the healthy individuals' subsample (who are presumed carriers of disease liability but are not affected/medicated).

Methods

Sample

The total sample consisted of 99 subjects from 26 families, each with an offspring diagnosed with EOBD (here defined as before 18 years). There were 21 four-member families (father, mother, patient and sibling) and 5 three-member families (father or mother, patient and sibling).

Families were recruited at the Child and Adolescent Psychiatry and Psychology Department of Hospital Clínic, Barcelona, Spain. The inclusion criteria for the offspring were BD diagnoses (type I or II), between 12 and 18 years of age, a Beck Depression Inventory raw score of < 18, and

a Young Mania Rating Scale raw score of < 8. The exclusion criteria were not-otherwise-specified (NOS) forms of BD, schizoaffective disorders, eating disorder patients with low weight (body mass index < 17), drug-dependence disorder or current drug abuse, drug-induced psychosis, autism spectrum disorder, organic or neurologic diseases, and intellectual disability.

The diagnoses, both in patients and relatives, were established according to DSM-IV criteria by two clinical psychiatrists (LL and RC). In cases, the principal diagnosis and comorbidities were confirmed by Kiddie-SADS-PL [52] and the treatment was provided in accordance with internal guidelines, based on the recommendations of the American Academy of Child and Adolescent Psychiatry [53]. In parents and adult siblings, lifetime diagnoses and personal psychiatric history and treatment were assessed by means of a clinical interview. Parents were also interviewed regarding younger siblings' psychopathology and previous treatments.

Neuropsychological evaluation was carried out by a clinical psychologist (SL) once patients had been in a state of euthymia for 1–3 months.

Measurements and instruments

The cognitive performance of all subjects was assessed with the same battery of neuropsychological tests, as detailed in Table 1. The mean raw scores of each test for each group are shown in Supplementary Table 1 (all sample) and Supplementary Table 2 (subset of patients and healthy relatives).

Given that most of the instruments do not have standardized scores for individuals under the age of 16, all measurements recorded were transformed to *z*-scores according to the mean and standard deviation of each sample subgroup, namely patients, parents and siblings. A theoretical approach based on traditional supported dimensions [26] initially guided the reduction of all measurements into a model consisting of four cognitive dimensions: reasoning skills, attention and working memory, memory, and executive functions (Table 1). At first, we considered all the variables of each instrument according to their description in the published manuals and we calculated the intra-class correlations of each dimension. Later, some of the initially included measurements were removed to improve the intraclass correlation of the dimensions.

The final dimensions were as follows:

The reasoning skills (RS) dimension. Although the mean between block design and vocabulary of Wechsler scales has been traditionally used as an estimation of intelligence quotient, their intra-class correlation was weak in our sample (ICC = 0.37). Accordingly, we decided to enrich this dimension with other three variables that measure skills based on crystalized thinking and fluent reasoning: Semantic fluency from COWAT and the number of categories and conceptual level from WCST (Table 1). The correlation between these three variables and the traditional estimation of intelligence achieved a substantially higher correlation (ICC = 0.64).

The memory (ME) dimension. This included short- and long-term measurements of visual and verbal memory and learning (Table 1) and showed a high intraclass correlation index (ICC = 0.87).

The attention and working memory (AW) dimension. This included measurements of auditory or visual focusing and selective attention, and measurements of auditory working memory (Table 1). It showed a high internal consistency index (ICC = 0.87).

The executive function (EF) dimension. This included set-shifting measurements (Table 1) and also showed a high intraclass correlation index (ICC = 0.87).

Statistical modelling

All statistical analyses were implemented with Stata v. 14 (StataCorp, 2013).

For descriptive purposes, the individual raw cognitive scores between patients, parents and siblings are displayed in Supplementary Table 1 (all sample) and Supplementary Table 2 (subset of patients and healthy relatives). They were compared by means of linear mixed models with the cognitive scores as dependent variable, familiar status (patient, parent or sibling) as a fixed-effect factor, sex and age as fixed covariates and family as a random effect factor (subjects nested with families).

The main analyses followed three steps (Fig. 1):

(1) Familiality analysis, which was conducted for the four cognitive dimensions separately, first in the total sample and second in the healthy relatives' subsample. This analysis reports a sample-based family aggregation estimation for each cognitive dimension (see below "intraclass correlation coefficient"). Familiality was analysed by means of a two-level linear mixed model (LMM) with the dimension z-score as the dependent variable, patient status (in the total sample analysis only), sex and age as fixed-effect covariates, and family as the random effect (subjects nested within families). The familiality of a cognitive dimension was documented if the variance of the random effect of family was significantly greater than zero. Then, the strength of the familial effect was measured by calculating the family-level residual intraclass correlation coefficient (ICC). The ICC score ranges from 0 to 1, with 0 suggesting no aggregation and 1 complete familial aggregation for the trait.

(2) Intra-family resemblance score (IRS) calculation, which was done only for those dimensions that were detected to be significantly familial in the step (1). It is calculated both in the total sample and in the healthy relatives' subsample. This reports a quantitative family-based value; i.e.

Table 1 Description of the neuropsychological measures included in each of the cognitive dimensions and the instruments that were used for their assessment

Dimension	Measures	Instrument
Reasoning skills (RS)	**Block design:** A visual-spatial intelligence measurement in which the subject is asked to reproduce a design with blocks **Vocabulary:** A measurement of crystalized intelligence in which the subject is asked to describe the meaning of several words In both tests, a higher score is indicative of better performance	Wechsler intelligence scale for children 4th edition, WISC-IV (< 17 years old) [98] Wechsler adult intelligence scale 3rd edition, WAIS-III (≥ 17 years old) [99]
	Semantic fluency (animals): A task of verbal fluency in which the subject is asked to evoke the maximum possible names of a concrete semantic category in one minute. It is a measurement of crystalized intelligence A higher score is indicative of better performance	Controlled oral word association test, COWAT [100]
	Number of categories: Through a visual association-related task, it measures logical reasoning and flexible thinking **Conceptual level:** This index suggests the visual association level of comprehension In both, a higher score is indicative of better performance	Wisconsin card sorting test, WCST [101]
Attention and working memory (AW)	**Digits:** The subject is asked to recall digits sequences in direct and reverse order **Letters and numbers sequencing:** The subject is asked to sort mentally mixed sequences according to ascending numeration and alphabetical order Both tasks provide measurements of selective attention and working memory In both, a higher score is indicative of better performance	Wechsler intelligence scale for children 4th edition, WISC-IV (< 17 years old) [98] Wechsler adult intelligence scale 3rd edition, WAIS-III (≥ 17 years old) [99]
	Detectability shapes/detectability 4-digits: They are measurements of the attention activation in divergent attention tasks In both, a higher score is indicative of better performance **Omissions shapes/Omissions 4-digits:** They are measurements of the orientation activation to the task In both, a higher score is indicative of worse performance	Continuous performance test-identical pairs, CPT-IP [102]
Memory (ME)	**Immediate and delayed logical memory:** The subject is asked to recall and verbally repeat two different stories, immediately after the presentation and 30 min later **Immediate and delayed visual reproduction:** The subject is asked to reproduce by drawing five different shapes, immediately after the presentation and 30 min later In all, a higher score is indicative of better performance	Wechsler memory scale, 3rd edition, WMS-III [103]
	Total hits list A: It is a measurement of the verbal learning capacity in which the subject is asked to recall and repeat a list of 16 disordered words but somehow related **Short-term free recall:** It is a measurement of verbal memory and recuperation capacity after the learning process in which the subject is asked to repeat the 16 words after an interference (another list of words) **Long-term free recall:** It is a measurement of verbal memory and learning consolidation capacity in which the subject is asked to repeat the 16 words 20 min after the learning process In all, a higher score is indicative of better performance	California verbal learning test, CVLT (in its Spanish version TAVEC) [104]

Table 1 (continued)

Dimension	Measures	Instrument
Executive function (EF)	**Total errors:** The number of total errors indicates the difficulty of the subject to understand the correct associations **Perseverative errors:** It is the number of errors due to set-shifting difficulties and suggests an inflexible style of thinking **Non-perseverative errors:** It is the number of errors due to random associations or difficulties to maintain the response to the correct associations In all, a higher score is indicative of worse performance	Wisconsin card sorting test, WCST [101]

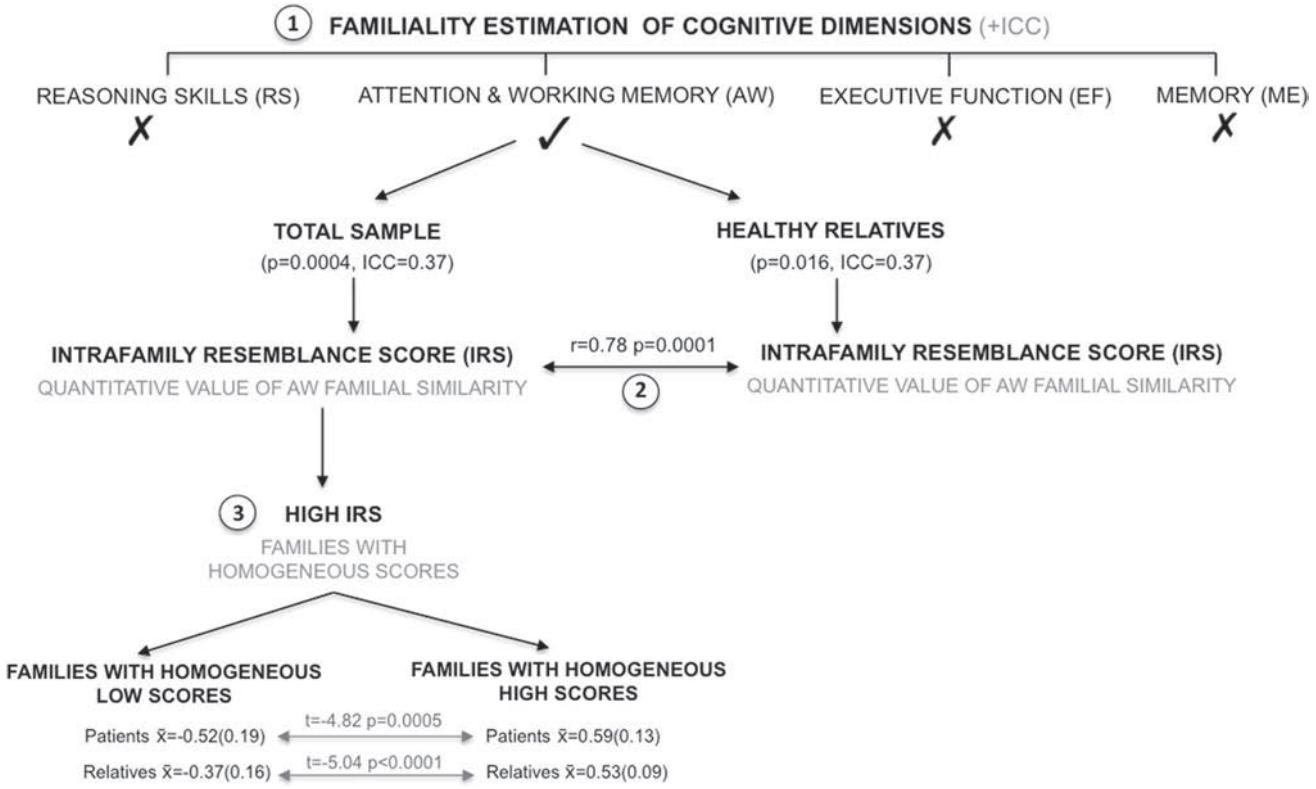

Fig. 1 Flowchart with the different analyses conducted as part of the study. 1. Familiality (intraclass correlation index, ICC) of the four cognitive dimensions was assessed in both the total sample ($n=99$) and the subsample of healthy relatives ($n=55$). 2. The IRS was estimated for those cognitive dimensions that showed familial aggregation (AW). The IRS was calculated in both samples (total sample and subsample of healthy relatives), and families were ranked accordingly. Both IRS were highly correlated. 3. We selected families with high IRS (IRS > 0). These families were dichotomised based on their mean family AW scores. Patients and relatives of the two generated groups differed in their AW scores

a value that is shared by all the family members, because it reflects the degree of similarity among them.

Based on the results obtained in step (1), we calculated the intra-family resemblance score (IRS) [54] for the AW dimension and we did it both in the total sample and in the healthy relatives' subsample. For each IRS calculation, we first created a dataset comprising all possible pairs of family members within each family, thereby including $N*(N-1)/2$ pairs for each family of N members. We then fitted a two-level LMM, with absolute differences in the cognitive dimension scores between the members of each pair as the dependent variable. Sex and age of the members in the pair were included as fixed-effect covariates, and family as the random effect factor. Since each family comprised only $N-1$ independent pairs, a family size weight of $2/N$ was applied in this model [55]. Finally, we calculated random

effect estimates (best linear unbiased predictions, BLUPs) for each family to obtain the IRS, a continuous parameter characteristic of each family that indicated the degree of intra-family similarity in the cognitive dimension scores after adjusting for the covariates effect. The IRS is, by definition, normally distributed with a mean of 0. Lastly, the IRS was multiplied by − 1 to facilitate interpretation: a higher IRS (i.e. > 0) indicates a greater resemblance among family members, whereas a lower IRS (i.e. < 0) indicates a lower resemblance.

(3) Interpretation of IRS by analysing its relationship with cognitive scores. The IRS represents the degree of similarity but does not inform on the direction of such similarity. In the case that families could only be homogeneous for low or high scores, we would observe a significant correlation between the IRS and the mean familial scores of a cognitive dimension. However, it is also plausible that some families show heterogeneous scores among their members. If that is the case, the more homogeneous patterns will correspond to those families with IRS > 0.

Accordingly, we first analysed the relationship between the IRS and AW mean familial scores by means of a linear regression. Afterwards, we applied a median-based binning method to dichotomise the families whose members showed more homogeneous AW scores (IRS > 0) based on their mean family AW scores. Finally, t test comparisons were used to analyse whether individuals of the families of the two generated groups differed in their AW scores.

Results

Sample

The total sample included 26 patients [51% men, mean age = 16.7(1.6)], 23 mothers [mean age = 47.2 (4.4)], 24 fathers [mean age = 49.6 (4.3)] and 26 siblings [50% males, mean age = 18.2 (5.1)].

As regards to patients, 21 (81%) had a diagnosis of BD type I and 5 of BD type II. Twelve (46%) had history of previous hospitalizations (9 had been hospitalized one time, 2 twice and 1 four times). Twenty-four cases had made a psychological consultation previously to the current affective episode [mean age = 9.3(4.0)] and 19, a previous psychiatric consultation [mean age = 13.0(3.7)]. The mean age of the beginning of affective unspecific symptoms was 10.9(3.9) and 13.0(3.8) for affective specific symptoms. The mean total scores of clinical questionnaires administered at neuropsychological assessment were 2.1(2.1) for YMRS and 8.1(5.1) for BDI.

All EOBD patients were receiving mood-stabilizing medication (14 lithium, 7 valproate, 2 topiramate, 1 carbamazepine, 1 oxcarbamazepine, 1 lamotrigine), 11 of them a co-adjuvant neuroleptics (4 risperidone, 4 olanzapine, 3 quetiapine), 3 cases were receiving antidepressants (2 sertraline, 1 fluvoxamine), and 3 more, low doses of benzodiazepines (1 clonazepam, 2 diazepam). The mean duration of drug treatment was 8.4(7.6) months for mood stabilizers, 14.5(12.3) months for neuroleptics, 4.4(7.2) months for antidepressants and 0.1(0.3) months for benzodiazepines.

Sixteen relatives (9 fathers, 5 mothers and 2 siblings) had a lifetime psychiatric disorder (parents: 4 with major depressive disorder, 4 with BD type I, 2 with substance abuse problems, 1 with eating disorder NOS, 1 with psychotic disorder NOS, 1 with anxiety disorder NOS and 1 with personality disorder NOS; siblings: 1 with major depressive disorder, 1 with eating disorder NOS). To define a sample subset only composed of healthy individuals (and to avoid the confounding effect of the disorder), these 16 relatives with a lifetime diagnosis together with the index cases of each family were excluded. To calculate familiality, it is necessary to have at least two family members per family, therefore, among the healthy individuals, those which were the unique remaining members of a family were also excluded. Then, the subset of healthy relatives was composed of 55 individuals, including 18 mothers [mean age = 47.11 (4.45)], 15 fathers [mean age = 49.80 (3.78)] and 22 siblings [59% males, mean age = 17.92 (4.71)]. 95% of families proceeded from urban areas. 40% of families had a high or middle–high economic status, 36% a middle status and 24% a middle–low or low status.

Almost all patients (96%) and all siblings were students, 19% of parents had a graduate degree, 70% were qualified workers or little businessmen and 11% were unqualified workers. As expected, parents had more years of education [17.7 (5.4)] than patients [13.04 (1.67)] and siblings [14.1 (4.4)]. No differences on years of education were found between the total sample of parents and the subset of healthy parents [17.70 (5.38) vs 17.37 (5.55)] and the total sample of siblings and the subset of healthy siblings [14.07 (4.34) vs 13.77 (3.94)].

Familiality and intra-family resemblance analyses

Familiality was detected for the attention and working memory (AW) dimension only (Fig. 1). The variance of the family random effect was significantly greater than zero in both the total sample (p value = 0.0004) and the subsample healthy relatives' subsample (p value = 0.016). ICC (95% CI) estimates showed that AW presented a moderate degree of familiality in both the total sample [0.37 (0.17–0.62)] and healthy relatives [0.37 (0.14–0.68)].

Once found that the AW dimension showed familial aggregation, the IRS was then calculated for this dimension both in the total sample and the sample of healthy relatives. Both IRS indexes were highly correlated (r = 0.78,

$p = 0.0001$). Accordingly, families could be ranked indistinctively according to the IRS obtained in the total sample or in the healthy relatives' subsample.

Next, for a better interpretation of the IRS indexes, we tested the association between IRS indexes and family mean AW scores. No significant linear relationship was observed (total sample: $\beta = 0.15$, SE = 0.02, $p > 0.05$; healthy relatives' subsample: $\beta = 0.07$, SE = 1.1, $p > 0.05$). These results indicate that intrafamilial resemblance is independent of the mean family AW scores, i.e. there are families whose members share either high or low AW scores and families with heterogeneous scores among their members.

Following our aim to identify a liability marker that can potentially help to identify more homogeneous subgroups of the disorder, we focused on families whose members showed similar performance on AW dimension. This was done by ranking the families according to their IRS calculated in the total sample and selecting those with an IRS > 0 ($n = 13$). It is of note that this IRS > 0 score represents homogeneity in AW performance among family members but does not inform on whether scores are similarly high or low. Accordingly, we used a median-based binning method to dichotomise these families according to the mean family AW scores and we analysed whether individuals of the families of the two generated groups differed in their AW scores. As regards to patients, individuals of the two groups show significantly different performance ($t = -4.82$, $p = 0.0005$): $\overline{X} = -0.52(0.19)$ ($n = 6$) vs to $\overline{X} = 0.59(0.13)$ ($n = 7$). A similar effect was observed when relatives belonging to the two groups were compared ($t = -5.04$, $p < 0.0001$): $\overline{X} = -0.37(0.16)$ ($n = 16$) vs $\overline{X} = 0.53(0.09)$ ($n = 20$).

Discussion

Despite the fact that some authors have suggested that more severe cognitive impairment could be a marker of increased vulnerability, a heterogeneous pattern of cognitive deficits among EOBD patients has been described [23–28]. The current study aimed to investigate whether such heterogeneity is a proxy of disease liability, by means of a family-based approach. We first examined the familial aggregation of four cognitive dimensions in families with an offspring with EOBD. Second, we estimated the intra-family resemblance score (IRS) to classify the families according to the level of cognitive similarity/dissimilarity among their members.

Regarding the first aim, we found that the attention and working memory (AW) dimension aggregated significantly in families with an offspring with EOBD, while this was not observed for the others (executive function, memory, and reasoning skills). The fact that familiality for this dimension was detected in both the total sample and the subset of healthy relatives, and that the degree of aggregation was the same in both cases, precludes the possibility that the aggregation observed was due to the disorder itself and, therefore, suggests that it may be considered as a familial vulnerability marker for EOBD. In this respect, the degree of familiality observed (ICC = 0.37) reflected a moderate influence of familial effects (i.e. genetic and environmental factors shared within families). This was consistent with previous studies that have reported that attention and working memory are genetically influenced. In this sense, estimates of the heritability of these cognitive dimensions are in the ranges of 0.15–0.72 and 0.28–0.79, respectively, in both healthy and clinical samples [56–60]. Accordingly, they have already been highlighted as promising intermediate phenotypes for BD [38, 61]. However, as Tirapu et al. (2017) reported, there is no consensus in the classification of attentional components [62]. Previous studies have supported the inclusion of a dimension that encompasses both selective/divided/sustained attention and working memory, [63] while others have considered that these aspects work online at a higher level of attention processing [64, 65].

Based on previous intermediate phenotypes studies on BD samples [44–48], we expected that the executive function, memory and the reasoning skills might show a degree of familiality. However, these dimensions did not show familial aggregation in our sample. Despite we cannot rule out the possibility of false negative results, it is important to consider that our sample differs from the previous studies in size and diagnose (EOBD). In addition, it should also be taken into account that the familial aggregation approach, as mentioned above, goes beyond the identification of differences among patients and relatives. Therefore, further studies are needed in larger EOBD samples also including controls, which would allow extending our study as well as comparing the within-family and the inter-group analyses. Also, the growing evidence of impairments in social cognition in BD [66–68] and its suggested influence on other cognitive dimensions and functionality [66, 67, 69], indicate that new studies including the social cognition dimension would be of interest for the better characterisation of EOBD cognitive performance.

Once we detected the familial aggregation of AW performance, we proceeded to estimate the IRS for this dimension in both the total sample and healthy relatives. The resulting classifications were highly correlated, once again indicating that this familial similarity is independent of disease status. Neither of the two IRS was linearly related to the mean AW scores of the family, thus indicating that the increase in intrafamilial similarity was not associated with an increase/decrease in the family mean AW score. To interpret this result, it is important to consider that a high IRS represents families whose members have similar scores, either high or low. Therefore, this non-relationship indicates that our sample was heterogeneous in terms of the degree of familial

resemblance for the AW dimension. In an attempt to better characterise the degree of within-families similarity, we observed that when families were dichotomised based on their mean AW dimension score, it was possible to differentiate families whose members had similar positive scores than those with similar negative scores on AW scores. Accordingly, despite the arbitrariness of the median-based binning method, the observed differences suggest that among families with AW homogeneous scores, there are families with different degrees of AW performance. This approach would be enhanced by, for example, clustering analysis; however, this was not possible due to the limited size of the sample in which this analysis was conducted. Accordingly, even though the interpretation should be done with caution, our data seem to be suggestive about the interest of the AW dimension as a familial liability marker, which could be putatively used to distinguish different familial subgroups with a differential genetic loading for attention and working memory deficits.

In relation to existing evidence, our results are consistent with previous studies that have defined specific subgroups of BD characterized by certain cognitive profiles [31–35, 70]. Generally, these studies converge on the identification of three main cognitive subgroups among patients: those with no cognitive deficits, those with generalized impairments and those with selective deficits in certain domains. It is worth mentioning that attention deficits are generally detected in all studies, in both subgroups (selective and generalized deficits).

In addition to these patient-based studies, family-based studies have also reported attention and working memory deficits in relatives [38, 42, 44, 71–73]. For example, Bora et al. (2009) showed a wide range of cognitive impairments in the first-degree relatives of patients with BD, including response inhibition, set shifting, executive function, verbal memory and sustained attention [38]. Lin et al. (2017) found that offspring with at least one parent with BD presented more deficits in working memory, visual-spatial memory and cognitive planning than healthy controls with unaffected parents [42]. Volkert et al. (2016) used a moderate sample size (27 patients and 27 relatives) and found that 70% of the patients showed cognitive deficits (defined by at least one test measurement 1.5 SD below the average of the normative data group), while the presence of deficits in relatives was observed in 56% [73].

Thus, previous family studies based mainly on the comparison of cognitive functions between groups of patients, relatives and healthy controls support the role of attention and working memory as BD vulnerability markers. As mentioned above, our results converge with the conceptualization of the attention and working memory as a liability marker in EOBD; however, the current within-family approach does not allow testing whether the observed performance is normative or, in other words, whether the vulnerability marker refers to cognitive deficits or not. Nonetheless, previous data from our research group point towards that several cognitive functions of at least part of the patients were impaired. In a previous study using a partially overlapping sample with the current one, we compared the cognitive outcome of 20 EOBD patients with 20 healthy controls after 2 years of follow-up [74]. EOBD patients showed greater than one standard deviation in several cognitive tasks (Block design, vocabulary, letter–number sequencing, total errors, non-perseverative errors and number of categories of WCST, and both immediate verbal and visual memories). In addition, in the current sample, patients have shown significant underperformance as compared to relatives in different cognitive tests (Supplementary Table 1 and 2).

Then, our study adds value to this above-mentioned evidence by focusing on the familial aggregation of four different cognitive dimensions in a sample of families with EOBD. It is of note that the study of the familiality of a trait has been successfully used to detect those phenotypes that maximize the phenotype–genotype correlation, as a first step in unravelling the molecular genetic underpinnings of specific symptoms or traits associated with psychiatric disorders. For example, this strategy has been applied to dissect the heterogeneity of major depressive disorder based on age at onset [75]. Also, another study reported that some catatonia-related phenotypes exhibited substantial familial aggregation, suggesting their use for subsequent molecular studies [76]. In addition, we have applied the IRS estimation as a strategy to go from a sample-based result (familiality) to a single-family characterization. Then, the strength of this approach lies on that it allows differentiating families with homogeneous cognitive performance and quantifying the degree of similarity among family members. This is in line with some other recent studies focused on schizophrenia, which have also proposed the interest of within-family approaches to identify disease-related genetic and environmental factors on several clinical and cognitive domains. For instance, by means of the IRS estimation, our group [54] described that schizotypy aggregation pattern in relatives might be predictive of patients' clinical characteristics. Also, a study based on a similar approach but based in a larger sample of patients with schizophrenia and their unaffected relatives described that patients' cognitive performance predicted significantly the relatives' scores [77].

In all, this approach can contribute to dissect the clinical heterogeneity of psychiatric disorders and our data particularly suggest that the AW dimension might be a familial liability marker for EOBD that could be used to distinguish different familial subgroups. Then, we could hypothesize that the different familial patterns in relation to the AW dimension might be influenced by genetic differences, among other factors. Therefore, the different degrees of aggregation

could be related to the convergence of genetic risk factors underlying the neurobiological mechanisms that regulate this dimension. In this regard, both adult-onset and early-onset BD [78–84] and cognitive functions [85–88] are highly polygenic phenotypes influenced by many common genetic variants, each of which exerts only a very small effect on the phenotypic variation. Despite the fact that some genome-wide association studies (GWAS) performed in adult BD samples have found no correlations between the disorder and cognitive abilities [87–90], other studies have demonstrated that the genetic variants that increase the risk for BD are also associated with cognitive functions [78, 91–95]. Interestingly, Hill et al. (2016) used GWAS data to test genetic correlations between general cognitive function (in childhood and older age), educational attainment and major psychiatric disorders [96]. The authors provided evidence that genetic variants associated with BD were correlated with polymorphisms related to educational attainment, but not with those involved in childhood cognitive ability (probably due to the sample size, as the authors suggested). As childhood cognitive function showed a large genetic correlation with educational attainment (rg = 0.719), the results of this study might suggest that cognitive function before the age of 30 is influenced by some of the genetic variants that are also involved in the onset of BD. Therefore, these data, together with our results, pose a challenge to test larger samples to identify whether the risk or protective genetic variants would be aggregated in homogeneous families with poorer or better AW, respectively.

Some limitations of this study must be acknowledged. First, the sample size and the lack of a replication sample represent the main limitations. However, it is important to consider the difficulty of sampling families with EOBD patients, as well as the fact that the limited number of EOBD families is detrimental to achieving a more homogeneous group with a higher genetic risk loading [9–11]. Also, the inclusion of a sample of control families could help to clarify whether the observed aggregation is specific of EOBD or reflect the transmission of AW abilities in families. Second, experts are not in full agreement about the factorial structure of cognitive functions in healthy subjects, and even less so between mental disorders. The MATRICS Consensus Cognitive Battery (MCCB) was originally created to systematically assess cognitive functioning in adult patients with schizophrenia, and was later exported to evaluate adults with BD, in which it showed good psychometrics [97]. Nevertheless, it has not been validated for use in younger samples with this disorder. Therefore, the theoretical model used to test cognition in our sample, supported by empirical data, cannot be representative of other families with offspring diagnosed as having EOBD [98–104].

Third, despite the fact that all patients were treated with mood stabilizers, this study did not take the specific pharmacological treatment of patients into account. However, as the AW dimension aggregated in the total sample and in the subset of healthy relatives, and the degree of aggregation was the same in both cases, it might indicate that the effect observed is independent of disease status (and, therefore, independent of treatment). The application of the familiality and IRS methodology in larger EOBD samples is needed to test the validity of the results of this study in independent samples. In addition, the use of larger samples would allow the genetic comparison of the two groups of families with low and high AW scores.

In conclusion, our results support the notion that the investigation of factors underlying the familiality of the AW dimension might represent a valuable strategy to define aetiological subgroups of families with EOBD, which could lead to more success in the search for the genetic underpinnings and mechanisms that underlie the disorder.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval Ethical approval was obtained from the local research ethics committees. All participants provided written consent after being informed of the study procedures and implications. All procedures were carried out in accordance with the Declaration of Helsinki.

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Supervisor's report on the contribution of the PhD applicant to the article

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as the supervisors of the present doctoral thesis by Jordi Soler Garcia, hereby certify that the participation of the PhD applicant in the article "Familial aggregation analysis of cognitive performance in early-onset bipolar disorder" included the following tasks:

- Conception and design of the study
- Statistical analyses
- Writing of the first manuscript draft
- Critical revision of the article for intellectual content

Dr Mar Fatjó-Vilas and Dr Lourdes Fañanás

Barcelona, May 14th 2021

3.

Influence of DAOA and RGS4 genes on the risk for psychotic disorders and their associated executive dysfunctions: A family-based study.

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Original article

Influence of *DAOA* and *RGS4* genes on the risk for psychotic disorders and their associated executive dysfunctions: A family-based study

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ABSTRACT

Background: Glutamatergic neurotransmission dysfunction has classically been related to the aetiology of psychotic disorders. A substantial polygenic component shared across these disorders has been reported and molecular genetics studies have associated glutamatergic-related genes, such as *D-amino acid oxidase activator (DAOA)* and *regulator of G-protein signalling 4 (RGS4)* with the risk for psychotic disorders. Our aims were to examine: (i) the relationship between *DAOA* and *RGS4* and the risk for psychotic disorders using a family-based association approach, and (ii) whether variations in these genes are associated with differences in patients' cognitive performance.
Methods: The sample comprised 753 subjects (222 patients with psychotic disorders and 531 first-degree relatives). Six SNPs in *DAOA* and 5 SNPs in *RGS4* were genotyped. Executive cognitive performance was assessed with Trail Making Test B (TMT-B) and Wisconsin Card Sorting Test (WCST). Genetic association analyses were conducted with PLINK, using the transmission disequilibrium test (TDT) for the family-based study and linear regression for cognitive performance analyses.
Results: The haplotype GAGACT at *DAOA* was under-transmitted to patients ($P = 0.0008$), indicating its association with these disorders. With regards to cognitive performance, the *DAOA* haplotype GAGGCT was associated with worse scores in TMT-B ($P = 0.018$) in SZ patients only. *RGS4* analyses did not report significant results.
Conclusions: Our findings suggest that the *DAOA* gene may contribute to the risk for psychotic disorders and that this gene may play a role as a modulator of executive function, probably through the dysregulation of the glutamatergic signalling.

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1. Introduction

Schizophrenia (SZ) is a severe psychiatric disorder that affects about 1% of the population worldwide [1], a figure that increases to 3.5% when schizophrenia-spectrum disorders (SSD) and bipolar disorders (BPD) are taken into account [2]. There are several epidemiological and clinical characteristics common to these diagnoses [3], and the evidence available also shows that generalized cognitive deficits are present across SSD and BPD, even though there may be quantitative differences [4].

Epidemiological and molecular genetic studies have demonstrated the genetic component of psychotic disorders. Twin and family studies have estimated that the heritability of SZ is between 65% and 80% [5]. Moreover, genome-wide association studies (GWAS) have reported a substantial polygenic component that contributes to the risk for the disorders [6,7]. These approaches have also highlighted the genetic overlap between SZ and BPD that has recently been quantified at around 15% (based on the correlation between SNP heritability estimates in both disorders) [8]. More specifically, since several of the genes associated with these disorders are involved in synaptic plasticity or glutamatergic neurotransmission, it is likely that an alteration in these processes may be part of their aetiology [7,9]. This is consistent with the glutamatergic hypothesis, which posits that NMDAR hypofunction contributes directly to negative symptoms and neurocognitive dysfunction and to positive dysfunction via the dysregulation of dopamine [10].

The *D-amino acid oxidase activator* gene (*DAOA*) and the *regulator of G-protein signalling 4* gene *(RGS4)* have been considered candidate genes because their encoded proteins play a role in regulating glutamatergic neurotransmission, and it is therefore hypothesized that they contribute to the genetic liability continuum described between psychotic disorders [11].

DAOA (13q33.2) encodes the protein DAOA, which activates D-amino acid oxidase (DAAO) in the brain, an enzyme that oxidizes D-serine, an important co-agonist for the *N*-methyl-D-Aspartate receptor (NMDAR) [12]. Substantial evidence indicates that *DAOA* could be involved in the pathophysiology of psychotic disorders. For example, SZ patients have been shown to have lower D-serine levels in serum [13] and cerebrospinal fluid [14] than healthy controls, while increased DAOA expression has been reported in the pre-frontal cortex (PFC) [15]. D-serine treatment has also been reported as having a beneficial effect on the negative symptoms of SZ [16].

RGS4 (1q32.2), which is expressed in the neocortex [17], is a member of a protein family that plays a crucial role in modulating signalling through G-protein pathways and acts as a GTPase activator by accelerating the hydrolysis of the guanine triphosphate (GTP) to guanine diphosphate (GDP) [18]. This reaction shortens the signal transduction duration of many neurotransmitters involved in psychotic disorders, such as dopamine, glutamate, serotonin and γ-aminobutyric acid (GABA) [19]. Decreased RGS4 protein levels have been detected in the PFC of SZ patients compared to healthy subjects [18,19].

Moreover, different studies have reported a genetic association of *DAOA* [e.g. 11,19–21] and *RGS4* [e.g. 22–24] genes, in both SZ and BPD, and a meta-analysis provided evidence about the role of the *DAOA* gene in both disorders [26].

In line with the above, we hypothesized that common genetic variants located in the genes involved in the homeostatic signalling system that regulates and stabilizes the efficiency of glutamatergic synapses, such as *DAOA* and *RGS4*, are associated with the risk of developing psychotic disorders, with no specificity among the different diagnoses. Furthermore, taking into account that i) deficits in this neurocognitive domain are central to both SZ and BPD [4] and, ii) they are already present in adolescents at risk of developing these disorders and in patients' first-degree relatives [27,28], we also hypothesized that these genes exert a modulating effect on patients' executive performance.

To test these hypotheses we examined the relationship between single nucleotide polymorphisms (SNPs) in *DAOA* and *RGS4* genes and the risk for psychotic disorders using a family-based association approach. Secondly, we investigated whether genetic variability in these genes was associated with differences in cognitive performance in patients.

2. Methods

2.1. Sample

Patients included in this study were drawn from consecutive admissions to three child and adolescent psychiatry units and two adult psychiatric units and evaluated by experienced psychiatrists from each unit. They all met the DSM-IV-TR diagnosis criteria for psychotic disorders, including schizophrenia, schizophreniform disorder, schizoaffective disorder, psychosis disorder not otherwise specified and bipolar disorder with psychotic symptoms. Exclusion criteria included major medical illnesses that could affect brain function, substance-induced psychotic disorder, neurological conditions and history of head trauma with loss of consciousness.

Patients were diagnosed based on the following schedules: K-SADS [29] for patients up to 17 years of age and CASH [30] for adult patients. Age at onset of the first psychotic symptoms was determined by means of the K-SADS and/or the SOS inventory [31].

Patients' parents and siblings were also recruited in line with the same exclusion criteria used for the patients. All underwent a clinical interview in order to evaluate their present and lifetime history of mental illness and/or treatment with psychotropic medication.

All participants provided written consent after being informed of the study procedures and implications. In the case of patients below the age of 18, written informed consent was also obtained from their parents or legal guardians. The study was performed in accordance with the guidelines of the institutions involved and approved by the local ethics committee of each participating centre. All procedures were carried out in accordance with the Declaration of Helsinki.

In a total sample of $n = 753$ Spanish Caucasian individuals from 222 families, two approaches were used: i) a family-based study, and ii) a case-based cognition study.

2.2. Cognitive assessment

Cognitive performance was evaluated in patients. This included the evaluation of executive function with the Wisconsin Card Sorting Test (WCST) [32] and the Trail Making Test B (TMT-B) [33]. The number of perseverative errors in WCST and the seconds required to complete TMT-B were selected as outcome measures. Cognitive assessment was carried out by experienced neuropsychologists and conducted when stabilization of symptoms and readiness for cognitive evaluation were decided by the clinical team.

2.3. Molecular analyses

Genomic DNA was extracted from peripheral blood cells using the Real Extraction DNA Kit (Durviz S.L.U., Valencia, Spain) or from buccal mucosa by means of a cotton swab using the BuccalAmp DNA Extraction Kit (Epicentre® Biotechnologies, Madison, WI).

Table 1
Information on *DAOA* and *RGS4* SNPs genotyped in this study.

	dbSNP	Gene	Genomic position	Region	Distance from previous SNP	Alleles[a]	MAF[1]	MAF[2]
SNP1	rs3916965	*DAOA*	106103360	5′ Intergenic	0	G/A	0.386	0.392
SNP2	rs3916967	*DAOA*	106117348	Promoter	13988	A/G	0.386	0.396
SNP3[b]	rs2391191	*DAOA*	106119446	Exon 2	2098	G/A	0.386	0.394
SNP4	rs778294	*DAOA*	106142235	Intron 3	22789	G/A	0.227	0.259
SNP5	rs3918342	*DAOA*	106185749	3′ Intergenic	43514	C/T	0.469	0.464
SNP6	rs1421292	*DAOA*	106198235	3′ Intergenic	12486	T/A	0.378	0.433
SNP1	rs1507754	*RGS4*	163025228	5′ Intergenic	0	A/G	0.452	0.438
SNP2	rs10917670	*RGS4*	163032842	Promoter	7614	C/T	0.436	0.436
SNP3	rs951436	*RGS4*	163033342	Promoter	500	A/C	0.427	0.452
SNP4	rs951439	*RGS4*	163033691	Promoter	349	C/T	0.436	0.425
SNP5	rs2661319	*RGS4*	163039777	Intron 1	6086	A/G	0.429	0.431

Data based on UCSC Human Genome Browser Feb. 2009 (GrGh37/hg19 Assembly). Minor allele frequency: MAF[1]: from the 1000 Genomes Project, MAF[2]: from our sample.
[a] The less frequent allele (minor allele) is placed second.
[b] Implies amino acid change (Arg30Lys).

Coverage of the *DAOA* and *RGS4* genomic sequence and ~10 kb upstream and downstream was achieved by including several tag SNPs. The optimal set of SNPs was selected by using SYSNPs (http://www.sysnps.org/) with a minor allele frequency (MAF) > 10% and the pairwise tagging option (threshold of $r^2 = 0.8$). The physical position of SNPs and the previous positive results were also taken into account. In total, six SNPs within *DAOA* and five within *RGS4* were selected for screening (Table 1).

SNP genotyping was performed using the Sequenom iPLEX GOLD MassARRAY platform in accordance with the manufacturer's instructions (Sequenom, San Diego, CA) at the Spanish "Centro Nacional de Genotipado" CEGEN-PRB2-USC.

2.4. Statistical analyses

All data were processed using SPSS 20.0 software (IBM SPSS Statistics for Windows, IBM Corp, Armonk, NY). The program Haploview v4.1 [34] was used to estimate the Hardy–Weinberg equilibrium.

Family-based associations were analysed with PLINK v1.07 [35] by means of the transmission disequilibrium test (TDT). TDT evaluates whether the transmission frequency of alleles from heterozygous parents to their affected children deviates from the expected Mendelian frequency by comparing the transmitted and non-transmitted alleles in a locus. Family-based analyses are more robust than case-control studies because they avoid population stratification problems and related spurious associations [36].

TDT analyses were run for both SNPs and haplotypes using a cut-off threshold for rare haplotypes of 1%. A sliding window approach was applied to the haplotype analyses.

Association analyses with cognitive measures were also conducted using PLINK, following a linear regression model (adjusted by age at interview, months of illness evolution and years of education). Haplotypes with a frequency > 10% were included in these analyses.

To test the diagnosis specificity of the results, both risk and cognition analyses were conducted in the complete sample and in two subsets: schizophrenia (SZ) and non-schizophrenia patients (non-SZ).

The genetic power of our family sample was calculated using Quanto 1.2. Given that the lower minor allele frequency observed in our sample was 0.259, all markers had 80% power to detect a risk effect with an OR ≥ 1.51 and a protective effect with an OR ≤ 0.63.

As regards multiple testing corrections, a 1000 permutations procedure was applied to all analyses and all the *P*-values given are those obtained with this correction. Moreover, considering that two genes were analysed and that different tests were developed (including diagnosis specificity tests and cognitive performance tests), a significance *P*-value threshold was established at 0.0028 (0.05/18).

3. Results

3.1. Sample characteristics

The sample characteristics are described in Table 2. Distribution of patients' DSM-IV-TR diagnoses was as follows: 50% schizophrenia, 15% psychosis NOS, 15% bipolar disorder, 12% schizophreniform disorder and 8% schizoaffective disorder.

3.2. Genetic association study

The genotyped SNPs included in this study are indicated in Table 1. The genotyping rate for each of the 11 genotyped SNPs was > 98.1%. None of the genotype frequencies significantly deviated from the Hardy–Weinberg equilibrium ($P > 0.05$). The observed minor allele frequency (MAF) of all SNPs was similar to those reported by the 1000 Genome Project [37].

3.2.1. DAOA *gene*

The haplotype GAGACT (from SNP1 to SNP6) was significantly associated with the disorders [$n = 222$, transmitted (T) = 22.92, untransmitted (UT) = 51.7, $\chi^2 = 11.1$ $P = 0.0008$, OR ($CI_{95\%}$) = 0.39 (0.23–0.69); haplotype frequency = 11%]. Given that this haplotype was significantly under-transmitted to patients, it can be assumed that it confers a protective effect.

Table 2
Demographic and cognitive characteristics of the family-based study sample.

	Patients ($n = 222$)	Parents ($n = 398$)	Siblings ($n = 133$)
Gender (male %)	71%	47%	41%
Mean age at interview	21.3 (6.6)	53.7 (9.08)	26.8 (6.85)
Years of education	11.2 (3.31)	12 (5.12)	13.4 (3.89)
Mean age at onset	19.6 (5.27)	–	–
Months of illness evolution	40.7 (50.3)	–	–
TMT-B (seconds)[c]	111.6 (55.6)[a,b]	–	–
WCST perseverative errors[c]	20.3 (15.1)[a,b]	–	–

Frequency (%) or mean (standard deviation) scores are given. TMT-B: Trail Making Test B; WCST: Wisconsin Card Sorting Test.
[a] There were no differences between SZ patient and non-SZ patient subsets in: (i) TMT-B: 113.54 (54.2) and 109.65 (62.3), respectively ($t = 0.24$ $P = 0.70$); (ii) WCST: 21.27 (16.1) and 19.38 (13.9), respectively ($t = 0.14$ $P = 0.87$).
[b] There were no differences between patients from childhood/adolescent and adult centres in: (i) TMT-B: 97.81 (70.1) and 115.29 (53.8), respectively ($t = -1.95$ $P = 0.052$); (ii) WCST: 19.19 (18.5) and 18.85 (14.5), respectively ($t = 0.14$ $P = 0.88$).
[c] Sample size was 198 for TMT-B and WCST.

Furthermore, diagnosis specificity analyses showed that, although not significant after correction, this haplotype was preferentially untransmitted to the subset of patients diagnosed with SZ [$n = 110$, T = 10.45 UT = 26.3, $\chi^2 = 6.8$ $P = 0.008$, OR ($CI_{95\%}$) = 0.33 (0.14–0.77); haplotype frequency = 12%] and to non-SZ patients [$n = 112$, T = 10.92, UT = 25.34, $\chi^2 = 5$ $P = 0.016$, OR ($CI_{95\%}$) = 0.36 (0.15–0.81); haplotype frequency = 8%].

Regarding the cognitive association approach, the haplotype GAGGCT was marginally associated with worse scores in TMT-B, specifically in SZ patients ($n = 100$; $\beta = 29.5$ $P = 0.018$, haplotype frequency = 12%). This association was not detected in non-SZ patients.

3.2.2. RGS4 *gene*

The haplotype TTGGA of *RGS4* was found to be under-transmitted to patients (complete sample) [T = 6.06, UT = 16.3, $\chi^2 = 4.68$ $P = 0.030$, OR ($CI_{95\%}$) = 0.33 (0.12–0.98); haplotype frequency = 3.1%]. However, this result did not remain significant after multiple test correction. Regarding the cognitive association approach, no significant association was detected between haplotypes and cognitive performance.

4. Discussion

The present study shows the association between haplotypes at *DAOA* and *RGS4* and the risk for developing psychotic disorders in a family-based study. This study also reports evidence of the involvement of the *DAOA* gene in the executive dysfunctions characteristic of patients affected by these disorders.

With regards to *DAOA*, the haplotype GAGACT was found to be associated with a protective effect, because the haplotype was under-transmitted to patients. Although marginal, this effect was also described separately in both SZ and non-SZ patient subsets, which suggests that *DAOA* may be associated with the psychotic disorder continuum in a non-diagnosis specific way. Although the risk haplotype identified in this study differs from some of those previously reported, our results are consistent with evidence of a genetic association between alleles in the *DAOA* gene and SSD [e.g. 11,19,20] and BPD [e.g. 21,25]. The observation of different associated alleles is, however, a relatively frequent phenomenon, and explanations for this primarily relate to differences in linkage disequilibrium, population stratification and different sample sizes [38,39]. Furthermore, clinical heterogeneity between samples is also an important factor to be considered.

Despite these discrepancies between studies, our results show similarities with some previous data. Firstly, with regard to particular *DAOA* SNPs in the haplotype described here, the A allele of rs778294 and the C allele of rs3918342 have previously been associated with a protective effect in both SZ and BPD [40,41]. Secondly, a recent study showed that SZ patients homozygous for the haplotype GAGA (SNP1–SNP4) experienced a larger increase in white matter volume in comparison to the rest of the sample [42]. Since loss of white matter volume has been described in both SZ and BPD [43,44], we could speculate that this haplotype, which is included in our haplotype, displays a protective effect in these disorders by modulating brain structure and/or functional phenotypes. In this regard, other previous neuroimaging studies have also reported that *DAOA* variants are associated with brain structure alterations in psychotic patients. For instance, Schultz et al. found that rs2391191 was associated with a distinct cortical thinning in SZ patients [45].

We have also added evidence of the role of *DAOA* in cognitive performance. We have found that genetic variation in this gene modulates executive function, with those SZ patients carrying the haplotype GAGGCT displaying a worse performance in TMT-B. Although this result was not significant after correction, it cannot be dismissed completely, since it is consistent with previous studies. Specifically, one haplotype within the *DAOA* gene has already been associated with a worse performance in TMT-B in SZ patients [46]. In addition, the rs2391191 has been significantly associated with episodic memory performance, as measured by the logical memory subtest of the Wechsler Memory Scale [47], whereas the rs3918342 and rs1421292 have been associated with attention, working memory and episodic memory performance, as measured by the Continuous Performance Test (CPT), N-back working memory test and the logical memory subtest of the Wechsler Memory Scale, respectively [48]. As occurred in the TDT approach, the haplotype we identified differed from those previously published. In addition to the aforementioned reasons, these differences might be explained by the use of different neurocognitive tests. Since both the WCST and TMT-B tests measure executive function, we would have expected the associated haplotype to have an effect in both tests. However, it is necessary to clarify that executive function refers to cognitive processes that involve a set of abilities that includes selecting and planning strategies, incorrect response inhibition, working memory and attention shifts. Therefore, although both tests are used to evaluate executive function, they are not completely overlapping measures and it is therefore possible that one SNP or haplotype had an effect in one test but not in the other. In this regard, in order to have two more homogeneous measures, it would have been interesting to use the Trail Making Test A (TMT-A), since a recent study suggested that the (TMT-B)–(TMT-A) score is a better indicator of executive function than TMT-B performance alone [49].

Another aspect of our findings that deserves attention is the fact that genetic variation in the *DAOA* gene was found to modulate TMT-B performance in SZ patients only. This specificity contrasts with the fact that we did not find differences in the TMT-B scores between the two patient subsets, although the SZ patients displayed worse mean scores than the non-SZ patients. According to the current view of perturbed executive function as an example of transnosological deficit that may be found in different diagnoses, including SZ and BPD [50,51], the reported lack of difference between groups in the present sample is not entirely unexpected. However, as mentioned above, executive function involves a subset of integrated subdimensions and TMT-B scores could reflect alterations in some of these. Therefore, although the SZ and non-SZ patient groups in our sample displayed non-statistically different TMT-B scores, each group could have a distinct impairment pattern [52]. Based on this argument, and given the polygenic basis of cognitive phenotypes [53], we suggest that the similarity in TMT-B scores between diagnoses could be the consequence of different disrupted subdimensions, and different genes could therefore be associated with executive function deficits [54].

With regards to the *RGS4 gene*, the family-based approach did not show any significant association with psychotic disorders, although there was a trend in the overall patient sample that suggested the haplotype TTGGA exerted a protective effect. Studies on the association between *RGS4* and psychotic disorders, especially SZ, report inconsistent results. While some studies have shown that *RGS4* is associated with SZ [23,24,55] and BPD [25], other studies have found no significant involvement of genetic variants within this gene in psychotic disorders [55–60]. Thus, although our results are consistent with some of those found in the literature, we cannot completely rule out the possibility of false negative results. Further studies are required in order to determine the role of this gene in the susceptibility for these disorders.

With regard to cognitive deficits, we could not find any association of *RGS4* with executive function. In addition, previous

studies have reported inconclusive results when tests for associations between *RGS4* variants and other cognitive endophenotypes were conducted. In that regard, Stefanis et al. did not detect any association between *RGS4* variants and cognitive phenotypes, except for the effect of one SNP on anti-saccade errors [61], a finding that Kattoulas et al. later replicated and extended to other oculomotor parameters [62]. Prasad et al. found an association between two SNPs (rs10917670 and rs951439) and face memory speed, but did not detect any association with any other cognitive domains [63], and So et al. detected an association between one SNP (rs951436) and verbal comprehension performance [64]. Hence, since there are no other studies in the literature assessing the association between *RGS4* and executive function, further studies are required in order to evaluate this gene involvement in the alterations of this cognitive domain.

Finally, in order to understand how changes in the gene sequence can impact the clinical and cognitive phenotypes studied – in this case, risk for psychotic disorders and executive dysfunctions–, it is essential to consider whether the variants analysed affect the final protein function or availability. In this regard, as explained in the introduction, SZ patients present higher *DAOA* expression in the PFC [15] and lower D-serine levels in serum and cerebrospinal fluid [13,14] than controls. In fact, it is plausible to suggest that an overexpression of *DAOA* could result in NMDR hypofunction by decreasing D-serine concentration. Therefore, genetic variability might contribute to modifying the expression, function and/or activity of the protein and, therefore, to explaining the observed differences between patients and healthy subjects. Among the genotyped SNPs in our study, only rs2391191 at the *DAOA* gene is non-synonymous (Arg30Lys) and therefore might be of putative functional relevance. However, there are no specific functionality tests for this SNP and no deleterious effect on the protein activity was found when its functional impact was predicted using PolyPhen-2 [65]. In spite of this, it is interesting to note that this non-synonymous SNP has been associated with reduced cortical thickness [45].

Moreover, recent data reveal the importance of intronic or intergenic variants as regulatory elements of gene expression [66]. Regarding the SNPs included in this study, to our knowledge, no data are available in the literature concerning the consequences of these non-coding variants on protein functionality or expression. In addition, the use of the bioinformatics tool HaploReg [67] in order to predict the effect of these SNPs on gene expression did not provide information about their possible regulatory role. Therefore, in order to draw a path between the gene sequence and the clinical and cognitive phenotypes, we could speculate that the variants located in the gene promoter alter the expression of the protein by modifying transcription factor binding sites, whereas the intronic variants could modify the function of the protein by disrupting the regulation of splicing, translation or mRNA polyadenylation processes.

Some of the limitations of this study must be acknowledged. Firstly, since only six and five polymorphisms at the *DAOA and RGS4* locus were respectively selected for analysis, they do not represent the whole gamut of these genes. Secondly, the antipsychotic treatment was not specified and, therefore, cognitive analyses, although covaried for age, months of evolution and years of education, were not adjusted for treatment type or duration. However, although the possible effect of medication on cognition is a potential concern, it should be noted that several pharmacological trials have reported that the action of antipsychotic drugs on neurocognitive improvement ranges from minor to neutral and there are no differences between treatments, including typical and atypical antipsychotic drugs [52,68]. Thus, although there are controversial findings in this area, this evidence suggests that medication probably did not have a major impact on the findings of this study. Thirdly, the moderate sample size and the lack of a replication sample were other limitations of this study. Finally, and linked with the previous limitation, after multiple testing corrections were addressed, some results did not remain significant. Although marginally significant results cannot be dismissed completely, since they come from a directional hypothesis and are partially in line with previous studies, they should be interpreted with caution, and replication studies are required.

Disclosure of interest

The authors declare that they have no competing interest.

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Supervisor's report on the contribution of the PhD applicant to the article

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as the supervisors of the present doctoral thesis by Jordi Soler Garcia, hereby certify that the participation of the PhD applicant in the article *"Influence of DAOA and RGS4 genes on the risk for psychotic disorders and their associated executive dysfunctions: A family-based study"* included the following tasks:

- Conception and design of the study
- Statistical analyses
- Writing of the first manuscript draft
- Critical revision of the article for intellectual content

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Barcelona, May 14th 2021

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Genetic variability in Scaffolding Proteins and the risk for schizophrenia and autism spectrum disorders: a systematic review.

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Review Paper

Genetic variability in scaffolding proteins and risk for schizophrenia and autism-spectrum disorders: a systematic review

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Scaffolding proteins represent an evolutionary solution to controlling the specificity of information transfer in intracellular networks. They are highly concentrated in complexes located in specific subcellular locations. One of these complexes is the postsynaptic density of the excitatory synapses. There, scaffolding proteins regulate various processes related to synaptic plasticity, such as glutamate receptor trafficking and signalling, and dendritic structure and function. Most scaffolding proteins can be grouped into 4 main families: discs large (DLG), discs-large-associated protein (DLGAP), Shank and Homer. Owing to the importance of scaffolding proteins in postsynaptic density architecture, it is not surprising that variants in the genes that code for these proteins have been associated with neuropsychiatric diagnoses, including schizophrenia and autism-spectrum disorders. Such evidence, together with the clinical, neurobiological and genetic overlap described between schizophrenia and autism-spectrum disorders, suggest that alteration of scaffolding protein dynamics could be part of the pathophysiology of both. However, despite the potential importance of scaffolding proteins in these psychiatric conditions, no systematic review has integrated the genetic and molecular data from studies conducted in the last decade. This review has the following goals: i) to systematically analyze the literature in which common and/or rare genetic variants (single nucleotide polymorphisms, single nucleotide variants and copy number variants) in the scaffolding family genes are associated with the risk for either schizophrenia or autism-spectrum disorders; ii) to explore the implications of the reported genetic variants for gene expression and/or protein function; and iii) to discuss the relationship of these genetic variants to the shared genetic, clinical and cognitive traits of schizophrenia and autism-spectrum disorders.

Introduction

Schizophrenia and autism-spectrum disorders are neurodevelopmental psychiatric disorders that have a prevalence of approximately 1% and 2.5% worldwide, respectively,[1,2] and have profound human and economic consequences.

Schizophrenia and autism-spectrum disorders were nosologically separated in the *Diagnostic and Statistical Manual Mental Disorders,* third edition (1980).[3] However, evidence has been accumulating to suggest that they may partially overlap in their clinical, neurobiological, behavioural and cognitive features, and that they may have some common etiological roots.[4] Regarding their clinical expression, some authors have proposed that the negative symptoms of schizophrenia can be construed more broadly as deficits in social communication and motivation, which are also found in people with autism-spectrum disorders.[5] Similarly, the grossly disorganized or abnormal motor behaviour described in schizophrenia includes a number of signs and symptoms consistent with those of autism-spectrum disorders, such as repeated stereotyped movements, echolalia, unpredictable agitation and decreased interaction with or interest in one's environment.[5,6] The disorders also share some cognitive deficits[7–9]; in particular, deficits in social cognition have received much attention.[10–15] As well, there are brain structural similarities between these disorders. For instance, lower grey matter volume in the limbic–striato–thalamic circuitry is common to schizophrenia and autism-spectrum disorders,[16] and reduced volume and thickness of the insula have been found in patients with first-episode psychosis and in high-functioning patients with autism-spectrum disorders.[17] Similar alterations to the white matter integrity of the left fronto-occipital fasciculus have recently been found in patients with schizophrenia and in patients with autism-spectrum disorders.[18]

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In recent years, the field of molecular genetics has been uncovering evidence of an overlapping and complex polygenetic architecture for these disorders. Evidence suggests that studying pathways common to both may shed light on their pathophysiology and clinical heterogeneity.

Robust longitudinal and epidemiological studies have shown that 25% of people with childhood-onset schizophrenia have a history of a premorbid autism-spectrum disorder[19]; that the adult outcomes of children with atypical autism include psychotic disorders[20]; that autistic traits in infancy increase the risk for psychotic experiences later in life[21]; and that there is some co-occurrence of autism-spectrum disorders and psychotic disorders.[22,23] This overlap is further supported by family studies, which have reported that the presence of one of these diagnoses in a first-degree relative increases the risk of the other.[24–27] Similarly, schizophrenia is more common in parents of patients with autism than in parents of healthy controls.[24]

Twin studies have also recognized the important contribution of genetic factors to both schizophrenia and autism-spectrum disorders, with heritability estimates of h^2 = 64%–80%[28,29] and h^2 = 64%–91%,[30] respectively.

Over the last decade, molecular studies have contributed to our initial understanding of the complex genetic architecture of schizophrenia and autism-spectrum disorders, and later to identifying genes that are involved in both disorders. In this sense, it is currently accepted that an individual's genetic risk of schizophrenia or an autism-spectrum disorder can be attributed to either many common variants with a frequency of > 1% (single nucleotide polymorphisms [SNPs]), each conferring a modest level of risk (odds ratio = 1.1–1.5); or rare mutations with a frequency of < 1% (single nucleotide variants [SNVs] and copy number variants [CNVs]) that are usually associated with a larger penetrance on the phenotype (odds ratio > 2).[31,32]

The most recent studies to examine genome-wide SNPs contributing to these disorders have estimated that genetic variation from SNPs accounts for 23% and 17% of the variance in risk of schizophrenia and autism-spectrum disorders, respectively.[33] Based on the significant but small correlation between SNP heritability estimates in both disorders, the coheritability between them has been quantified at around 4%.[33] In this regard, genome-wide association studies have identified several SNPs associated with schizophrenia and/or autism-spectrum disorders.[34–37]

Meanwhile, genome-wide and microarray-based comparative genomic hybridizations have found that the CNV burden is also increased in patients with schizophrenia or autism-spectrum disorders compared with healthy controls.[38–41] For example, microduplications of 1q21.1 or 16p11.2 and deletions at 2p16.3, 15q11.2 or 22q11.21 have been reported in patients with schizophrenia and autism-spectrum disorders.[42] De novo gene-disrupting SNVs have also been found to occur at higher rates in patients with autism-spectrum disorders than in controls.[43–46] In schizophrenia, the initially reported increased rates of putatively functional mutations[47,48] have not been replicated in 2 larger studies,[49,50] but those later studies found that the enrichment of loss-of-function de novo mutations was relatively concentrated in genes that overlapped with those affected by de novo mutations in autism-spectrum disorders. In addition, an excess of de novo mutation was confirmed in an independent sample of patients with schizophrenia.[51]

With regard to the identification of specific genes involved in both schizophrenia and autism-spectrum disorders, findings from CNV and SNV studies have shown a notable consistency in some functionally enriched sets of genes. Genetic studies assessing common or rare variants show a certain convergence on reporting genes involved in glutamatergic synapse plasticity.[49,52–55] A structure located in glutamatergic synapses that has been associated with both disorders is the postsynaptic density (PSD).[50,56–61] For example, Bayés and colleagues found that mutations in 199 human PSD genes were involved in more than 200 diseases, half being nervous-system disorders.[61] That study suggested that impairments in PSD proteins might underlie psychiatric disorders and their associated cognitive, behavioural and clinical phenotypes, but no systematic review based on this hypothesis has integrated the molecular data generated across studies in the last decade. Another example has been provided by Purcell and colleagues, who, after analyzing the exome sequences of 2536 patients with schizophrenia and 2543 controls, reported that SNVs were significantly more frequent in cases than controls, and that these SNVs were especially enriched in the activity-regulated cytoskeleton-associated (ARC) complex of PSD.[50]

PSD proteins and pathophysiological hypotheses

The PSD is a specialized matrix located at the excitatory postsynaptic terminals with a dish-shaped aspect, a surface area of 0.07 μm^2 and a thickness of 30 to 40 nm on electron microscopy (Fig. 1A).[62] The PSD can also be described as a highly organized and dynamic macromolecular complex consisting of several hundred proteins that process, integrate and converge the excitatory glutamatergic synaptic signals on the nucleus. As a point of convergence for the glutamatergic signalling pathways with other neurotransmitter systems, the composition and regulation of the PSD is essential for ensuring normal synaptic neurotransmission and plasticity.[63,64]

The PSD is enriched with different membrane components, such as glutamate receptors, tyrosine kinases, G protein–coupled receptors, ion channels or cell adhesion molecules, which are assembled by cytoplasmatic scaffolding proteins.[65] Among the proteins that make up the PSD, studies have reported associations between genes coding for scaffolding proteins and both schizophrenia and autism-spectrum disorders, suggesting that variants in these genes might increase the risk of these disorders. For instance, recent studies have found that SNPs and CNVs in autism-spectrum disorders and schizophrenia are particularly concentrated in scaffolding genes and other PSD-related genes.[66,67] Other studies have indicated changes in the expression of scaffolding genes in schizophrenia and autism-spectrum disorders compared with healthy controls.[68,69] A recent study reported that gene-disrupting ultra-rare variants were more abundant in schizophrenia cases than in controls, and that these mutations were

particularly enriched in scaffolding genes and other PSD genes.[70]

Scaffolding proteins can be defined as molecular circuit boards that can organize a wide variety of circuit relationships between signalling proteins. More specifically, the main function of scaffolding proteins is to bring together 2 or more proteins to facilitate their interaction and functions, linked to critical roles in cellular signalling. This is possible because scaffolding proteins are composed of several protein–protein interaction modules, most notably the PSD-95/discs large/zona occludens-1 (PDZ) and Src homology 3 (SH3) domains.[71] Since scaffolding protein complexes are dynamic, they have the ability to change specific protein interactions to rapidly adapt to changing environmental requirements or diverse signalling cues.[72] This versatility is related to their modularity, which allows for recombination of protein interaction domains to generate variability in signalling pathways. Such properties are seen as a simple evolutionary solution to controlling the specificity of information flow in intracellular networks, generating precise signalling behaviours.[73]

Owing to their dynamic configuration, postsynaptic scaffolding molecules not only establish the internal organization of the PSD, allowing neurons to respond efficiently to stimuli, but they also regulate processes related to synaptic plasticity, such as glutamate receptor trafficking and signalling, and dendritic structure and function,[74,75] which critically determine the characteristics of excitatory synaptic transmission (Fig. 1B).

Disruption of scaffolding genes might alter the homeostasis of the PSD and contribute to the synaptic dysfunctions associated with schizophrenia and autism-spectrum disorders.[76] However, despite the potential importance of scaffolding proteins in these psychiatric conditions, no systematic review has addressed the integration of genetic and molecular data generated across studies.

The nature and function of the different families of scaffolding proteins included in this review, and the characteristics of the genes encoding them, are shown in Figure 1 and briefly summarized below.

The discs large protein subfamily

The discs large (DLG) subfamily is a group of proteins in the membrane-associated guanylate kinase (MAGUK) family, and consists of DLG1, DLG2, DLG3 and DLG4. These proteins have 3 PDZ domains in their N-terminus, which allow them to interact with a variety of binding partners in the PSD, such as glutamatergic receptors, as well as other cytoplasmic scaffolding proteins. The DLG proteins control the transmission of extracellular signals to downstream signalling molecules of the PSD and regulate the localization of glutamatergic receptors *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) at neuronal synapses and dendrites.[77] Moreover, they regulate the trafficking and clustering of ionic channels and the excitability of the presynaptic terminals, affecting the amount of neurotransmitter released.[78] Since their temporal

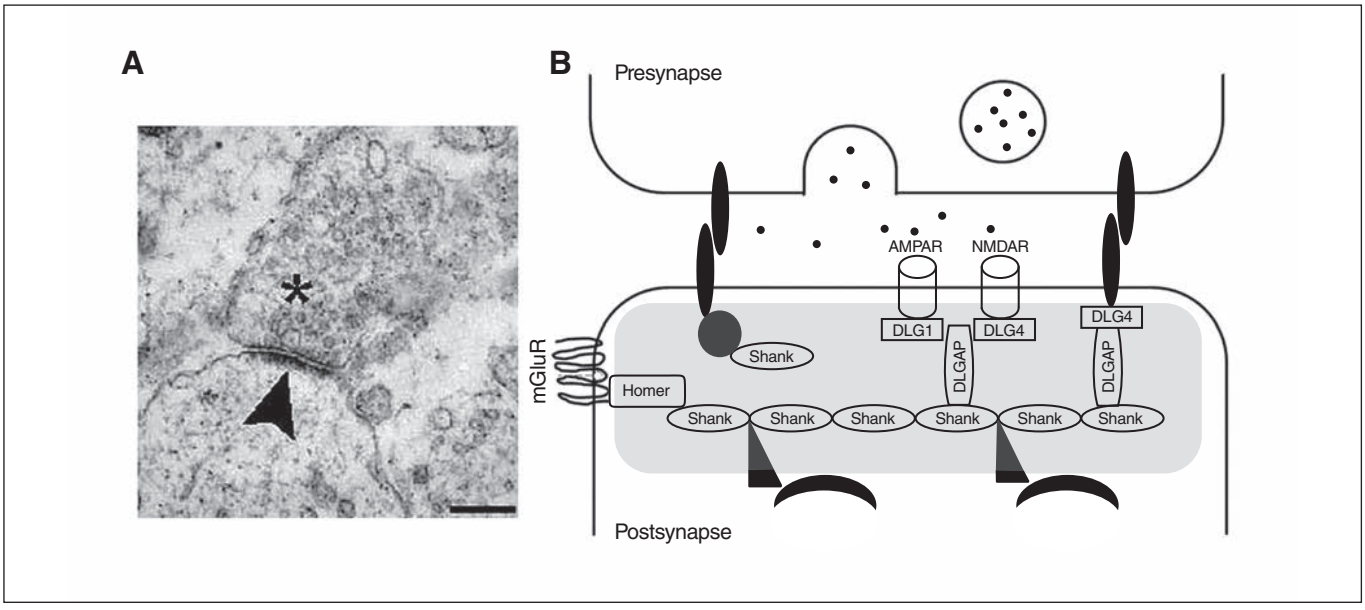

Fig. 1: Image of the postsynaptic density (PSD) and scheme of the scaffolding proteins at the PSD that have been analyzed in the present review. (**A**) An electronic microscope image of a synapse; vesicles can be observed in the presynaptic neuron (asterisk). The electron-dense structure observed in the postsynaptic element is the PSD (arrowhead). Scale bar, 250 nm. Image retrieved under the Creative Commons Attribution License from Heupel et al. *Neural Devel* 2008, https://doi.org/10.1186/1749-8104-3-25. (**B**) A scheme of the PSD (grey shading). Multimerization of Shank1 to 3 proteins generate a network that links numerous proteins to the postsynaptic receptors. Homer proteins, including Homer1b/c, Homer2 and Homer3, also act as adaptors and interact with several PSD proteins, such as type I-mGluRs. The DLGAP1 to 4 proteins interact with DLG proteins, including the DLG1/SAP-97, DLG2/PSD-93, DLG3/SAP-102 and DLG4/PSD-95, to coregulate different ion channels, such as the NMDAR and AMPAR. AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; DLG = discs large; DLGAP = discs-large-associated protein; mGluR = metabotropic glutamate receptor; NMDAR = *N*-methyl-D-aspartate receptor; SAP = synapse-associated protein.

and spatial expression differ, it is believed that DLG members complement each other in performing these functions from embryonic to adult stages.[79]

The *DLG1* gene (also known as SAP-97) maps on chromosome 3q29 and encodes the synapse-associated protein 97 (SAP-97 or DLG1), which is thought to play a role in synaptogenesis[80] and glutamatergic receptor trafficking during development.[77]

The *DLG2* gene (also known as PSD-93) is located on chromosome 11q14.1. Different studies have suggested that the protein it encodes (PSD-93, DLG2) plays a role in the regulation of synaptic plasticity. The DLG2 protein interacts with the tyrosine kinase Fyn, which is involved in the phosphorylation-based regulation of NMDA receptors that is required for the induction of NMDA-receptor-dependent long-term potentiation.[81]

The *DLG3* gene (also known as SAP-102) is located on Xq13.1, and the protein it encodes (DLG3) is the first protein related to intellectual disability that has been directly linked to glutamate receptor signalling and trafficking.[82] Later studies have replicated the association of this gene with intellectual disability,[83,84] suggesting that DLG3 somehow modulates cognition. This is consistent with the observed embryonic expression of this protein and its role in the regulation of synaptic formation and plasticity during brain development.[85]

The *DLG4* gene is located on chromosome 17p13.1, and the protein it encodes (PSD-95, DLG4) is involved in the maturation of synapse formation and the NMDA receptor signalling pathway**.** It participates in the clustering and trafficking of NMDA and AMPA receptors in the PSD.[63,79] Moreover, DLG4 interacts with the dopamine receptor D1 (DRD1) and the NMDA receptor, and regulates positive feedback between them.[86] The degradation of DLG4 is regulated by other proteins that have also been associated with autism-spectrum disorders.[87]

The discs-large-associated protein family

The discs-large-associated protein (DLGAP) family is made up of 4 proteins encoded by different homonymous genes: *DLGAP1* (18p11), *DLGAP2* (8p23), *DLGAP3* (1p34), and *DLGAP4* (20q11). All proteins have 5 repeats of 14 amino acids in the middle region, followed by a proline-rich sequence and a C-terminal PDZ-binding motif that mediate interactions with other PSD proteins.[88]

Although the differential roles of each family member are unknown, all DLGAP proteins play an important role in organizing the postsynaptic signalling complex in glutamatergic synapses,[89] and are especially involved in the stabilization of synaptic junctions and regulation of neurotransmission.[90] In addition, DLGAP proteins clearly have a central role in the regulation of synaptic ion channels, including both NMDA and AMPA receptors.[91]

DLGAP2 (also known as SAPAP2 or GKAP) is the most studied of the proteins in this family. It interacts directly with DLG4 and Shank proteins to form a complex that plays critical roles in synaptic morphogenesis and function.[90,92]

It has been proposed that SAPAP proteins provide a link between the PSD-95 family of proteins and the actincytoskeleton through interactions with the Shank/ProSAP proteins, which in turn bind the actin-binding protein cortactin.[93–97] Additionally, Shank/ProSAP also binds to Homer, which interacts with metabotropic glutamate receptors.[98] Therefore, in the current scaffolding model, PSD-95/SAPAP/Shank interactions play an important role in organizing the large postsynaptic signalling complex at glutamatergic synapses.[96,97,99,100]

The Shank protein family

Another PSD scaffolding protein group is the SH3 and multiple ankyrin repeat domains (Shank) family, which consists of 3 proteins encoded by different genes that are differently expressed in the brain[97,101]: *SHANK1* (19q13.33), *SHANK2* (11q13.3) and *SHANK3* (22q13.3). So far, it is unclear whether individual Shank family proteins fulfill unique physiologic functions, but the structural similarity between Shank forms has led to the observation that many interaction partners of Shank proteins in the synapse are recognized equally by all 3 family members.[93]

In this regard, Shank proteins crosslink Homer, DLGAP2 and DLG4 proteins in the PSD and participate in glutamatergic downstream signalling by assembling glutamate receptors with other scaffolding proteins, cytoskeleton factors and intracellular effectors.[102] Multimerization of Shank1–3 proteins can generate a network in the PSD that links numerous proteins to the postsynaptic receptors. In addition, Shank proteins promote the formation, maturation and enlargement of dendritic spines.[103]

The Homer protein family

Homer proteins include 3 different members that are encoded by 3 different homonymous genes: *HOMER1* (5q14), *HOMER2* (15q25) and *HOMER3* (19p13). Homer proteins can also be classified into constitutively expressed isoforms (i.e., Homer1b/c, Homer2 and Homer3), which are bimodal proteins with an N-terminal domain that mediates the interaction with other PSD proteins, and a C-terminal coiled-coil domain that enables self-assembly, as well as short splice variants (Homer1a and Ania-3) that lack the C-terminal domain and cannot self-assemble.[104] The various protein forms are differentially expressed over time and place.[105]

Homer proteins act as multimodal adaptors by interacting with several PSD proteins, such as type I metabotropic glutamate receptors (mGLuR1–5), Shank proteins or synaptic signalling molecules such as inositol 1,4,5-triphosphate receptors (IP3Rs), and binding them to the cytoskeleton.[102] Homer proteins are also involved in glutamatergic synapses by regulating glutamatergic receptor trafficking, the function of plasma membrane ion channels and intracellular messenger systems.[106] For these reasons, Homer proteins are important for cell signalling, cell excitability, synaptic neurotransmission and neuronal plasticity.[107,108]

Objectives

The objectives of this review were as follows: i) to conduct a systematic review of the literature in which variations within the above-mentioned scaffolding genes are associated

with either schizophrenia or autism-spectrum disorders, and to describe the degree of overlap between both diagnoses; ii) to explore whether the reported genetic variants putatively associated with schizophrenia or autism-spectrum disorders are involved in changes of gene expression or protein functionality according to basic research data; and iii) to consider the implications of the reported associations for the development of these disorders and their associated phenotypes.

Methods

We conducted a systematic search of the PubMed, PsycINFO and Web of Science databases. The search terms were "Schizophrenia or autism" and "postsynaptic density proteins or PSD or scaffold* proteins" without date restrictions. Inclusion criteria were original articles that reported i) the association of genetic variants (SNPs, SNVs and CNVs) of the genes in the DLG protein subfamily or the DLGAP, Shank or Homer protein families with schizophrenia or autism-spectrum disorders; and ii) genetic variations in these genes and their functional consequences, based on animal-model or in vitro studies.

This search initially retrieved 366 articles. After evaluating whether they fulfilled the inclusion criteria, we excluded 261 articles. Another 25 studies and reviews that were relevant for the topic were found from cross-referencing and included in this review. The final pool of articles comprised 130 papers (Fig. 2).

Results

Table 1 describes SNPs in scaffolding genes that have been associated with schizophrenia or autism-spectrum disorders. Table 2 describes SNVs, and Table 3 describes CNVs. Table 4 describes expression and functional information on scaffolding genes obtained from basic studies. Table 5 describes the SNPs, SNVs and CNVs of risk that are shared by both schizophrenia and autism-spectrum disorders.

DLG protein subfamily

Protein DLG1

Both SNPs in *DLG1* have been associated with schizophrenia (Table 1). An SNV has been associated with autism-spectrum disorders (Table 2). Furthermore, deletions of the chromosomal region 3q29 have been related to schizophrenia and autism-spectrum disorders (Table 3) and have been associated with impaired cognition and social dysfunction (Table 3).

Two studies reported reduced expression of *DLG1* in the prefrontal cortex of postmortem brain samples of patients with schizophrenia (Table 4). To the best of our knowledge, there are no expression studies in autism-spectrum disorders samples.

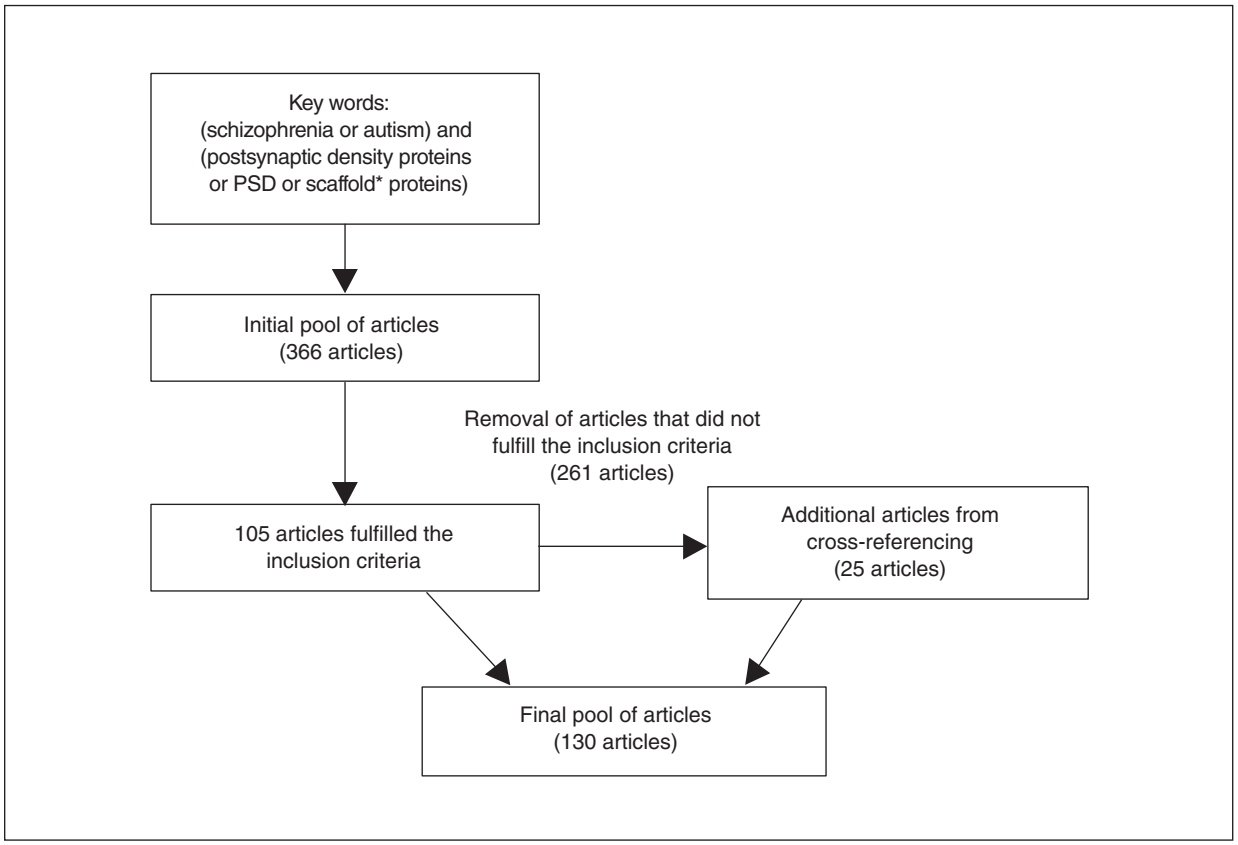

Fig. 2: Flow diagram of the literature search.

An animal-model study reported that glutamate-receptor NMDA antagonists upregulate *DLG1* mRNA expression in the cerebral cortex of mice (Table 4).

Protein DLG2

Different studies have identified rare mutations in the *DLG2* gene in schizophrenia and autism-spectrum disorders. While SNVs have been detected only in schizophrenia (Table 2), CNVs have been identified in both disorders (Table 3). Interestingly, a deletion identified in intron 6 of the gene in patients with autism-spectrum disorders[157] partially overlaps with another deletion spanning from intron 2 to intron 6 in patients with schizophrenia.[57]

Although still not tested in patients with autism-spectrum disorders, alterations in mRNA and protein expression have been reported in the prefrontal cortex of postmortem brain samples of patients with schizophrenia (Table 4).

Animal-model studies seem to support the function of DLG2 as a regulator of synaptic plasticity; they have shown that *DLG2* mutant mice display cognitive abnormalities and long-term potentiation deficits (Table 4).

Protein DLG3

To our knowledge, only 1 genetic study has associated 6 intronic SNPs in *DLG3* with autism-spectrum disorders (Table 1), although their consequences for protein function or expression are unknown.

Despite conflicting results in postmortem brain expression studies, it seems that alterations in *DLG3* could underlie the neurobiology of schizophrenia. In this regard, both increased and decreased *DLG3* mRNA and protein expression have been reported in the thalamus of schizophrenia patients (Table 4).

Animal-model studies support a role for this gene in cognition; mice lacking *DLG3* exhibit impaired learning (Table 4).

Protein DLG4

For the *DLG4* gene, SNPs (Table 1) and CNVs (Table 3) have been identified only in patients with schizophrenia and with

Table 1: Single nucleotide polymorphisms in scaffolding genes associated with schizophrenia, autism-spectrum disorders and other clinical phenotypes of interest

	Single nucleotide polymorphisms				
Gene	Schizophrenia	Austism-spectrum disorders	Schizophrenia and autism-spectrum disorders	Other associated phenotypes	Sources
DLG1	rs382579, rs2122824, rs7616588, rs7638423, rs6805929, rs2044862, rs4916461, rs338187, rs10489880[109] rs9843659[109,110]	—	—	—	Sato et al.[109] Uezato et al.[110]
DLG3	—	ss104807047, rs28391150, rs1886890, rs3215810, rs41303736, ss104807048[111]	—	rs28391150 (associated with intellectual disability)[83] ss104807047 (associated with intellectual disability)[84]	Zanni et al.[83] Philips et al.[84] Kantojärvi et al.[111]
DLG4	rs2230178, rs6145976, rs2017365, rs739669, rs13333[112] rs222837[113] rs390200, rs222853, rs17203281[112,113]	—	—	—	Cheng et al.[112] Balan et al.[113]
DLGAP1	rs145691437, rs3786431, rs201567254, rs3745051, rs11662259[114]	—	—	—	Li et al.[114]
DLGAP2	rs2906568, rs2293909[116]	—	rs2906569, rs2301963[115,116]	rs2301963 (associated with decreased orbitofrontal cortex white matter volume)[117]	Chien et al.[115] Li et al.[116] Wu et al.[117]
SHANK3	—	rs9616915[118,119] Independent studies did not find association with autism spectrum disorder[120,121]	—	—	Shao et al.[118] Mashayekhi[119] Sykes et al.[120] Qin et al.[121]
HOMER1	rs4704560, rs2290639[122] rs9293785[123]	—	—	rs4704560 C allele (associated with hallucinations in patients with Parkinson's disease)[124] rs2290639 (associated with substance abuse)[125]	Spellmann et al.[122] Zhao et al.[123] De Luca et al.[124] Strauss et al.[125]
HOMER2	rs2306428, rs86949, SNP20[126]	—	—	—	Gilks et al.[126]

SNP = single nucleotide polymorphism.

autism-spectrum disorders, respectively. In contrast, SNVs have been associated with both disorders (Table 2).

Among these variants, the SNP rs13331 (T/C), located at the 3′UTR of the gene, is especially interesting because the T allele was first associated with schizophrenia, and a posterior reporter gene assay indicated that subjects carrying this allele had decreased DLG4 protein activity. Based on these results, the authors suggested that reduced DLG4 activity or expression may increase the risk of developing schizophrenia.[112]

Alterations in mRNA and protein expression have been reported in postmortem brain samples of patients with schizophrenia (Table 4), although the direction of the results is inconsistent. Up to now, no expression studies have been performed in patients with autism-spectrum disorders.

Animal-model studies also appear to support an important role for DLG4 in regulating excitatory synapses and synaptic plasticity, while mutant mice also displayed clinical phenotypes related to schizophrenia and autism-spectrum disorders, such as impaired learning, abnormal

Table 2: Single nucleotide variants in scaffolding genes associated with schizophrenia, autism-spectrum disorders and other clinical phenotypes of interest (part 1 of 3)

	Single nucleotide variants				
Gene	Schizophrenia	Autism-spectrum disorders	Schizophrenia and autism-spectrum disorders	Other associated phenotypes	Sources
DLG1	g.196863463C>T[49] g.196812562A>T, g.196812614A>T, g.196842947T>C, g.196867096C>A[50] g.196863502G>C*[127]	g.196817764T>C[59]	—	—	Fromer et al.[49] Purcell et al.[50] Iossifov et al.[59] Xing et al.[127]
DLG2	g.83194295C>T[49] g.83180351C>T, g.83180371T>G, g.83243821T>G, g.83497759G>A[50]	—	—	—	Fromer et al.[49] Purcell et al.[50]
DLG4	g.7096826C>T[50]	g.7106562G>Aa[127]	—	—	Purcell et al.[50] Xing et al.[127]
DLGAP1	c.1922A>G[114]	—	—	—	Li et al.[114]
DLGAP2	g.1497520G>A[50] c.−69+9C>T, c.−69+13C>T, c.−69+47C>T, c.−69+55C>T, c.−32A>G, c.341A>G,* c.438C>T, c.990+60T>C, c.1192G>A, c.1920+37A>G, c.1920+94T>A, c.1927G>A, c.2493G>C, c.2634C>T, c.2797G>A,* c.2884G>A,* c.2663G>A[116]	c.44C>T,* c.277C>A,* c.545G>A,* c.574G>T, c.1516T>C,* c.2392G>C,* c.970A>T[115] g.1616734C>T*[127] g.1626547G>C[59]	c.841C>G,*† c.2135C>T,†‡ c.2750C>T*†‡[115,116]	—	Purcell et al.[50] Iossifov et al.[59] Chien et al.[115] Li et al.[116] Xing et al.[127]
DLGAP3	c.1141G>A, c.1759G>C, c.2309G>T, c.2578−11C>T[128]	35365700G>A[59]	—	—	Iossifov et al.[59] Li et al.[128]
SHANK1	g.51205733T>A[50]	g.51220161C>T, g.51206952G>A,* g.51205840C>T,* g.51170826G>A, g.51170775G>A, g.51165632C>T[129] g.51220076C>T, g.51219998C>T, g.51215287C>T,* g.51206988G<T, g.51205886C>T,* g.51191281C>T,* g.51172180G>A, g.51171270C>T,* g.51170856C>T, g.51170854C>T,* g.51170779C>T, g.51170674C>A,* g.51170418G>A, g.51170407G>T, g.51170362A>T,* g.51170359T>C,* g.51170046A>T,* g.51169830C>T,* g.51165932C>T, g.51165929C>T,* g.51165767G>A,* g.51165574C>A*[130]	—	—	Purcell et al.[50] Leblond et al.[129] Sato et al.[130]

Table 2: Single nucleotide variants in scaffolding genes associated with schizophrenia, autism-spectrum disorders and other clinical phenotypes of interest (part 2 of 3)

	Single nucleotide variants				
Gene	Schizophrenia	Autism-spectrum disorders	Schizophrenia and autism-spectrum disorders	Other associated phenotypes	Sources
SHANK2	g.70666649G>A, g.70666499C>A, g.70544817G>T, g.70349029T>C, g.70333526G>T, g.70333043G>T, g.70333967G>A, g.70331576C>T, g.70331462G>T, g.70319333C>A[131] g.70644595G>A*[132]	c.76C>T, c.622C>T, c.3380C>T, c.4048G>A, c.467A>G, c.492C>T, c.527−18C>A, c.640+11C>T, c.80033C>T, c.942+19G>A, c.924+133G>C, c.1061−81C>T, c.1141+49G>A, c.1148−109C>T, c.1201A>C, c.1264G>A, c.1302+35G>A, c.1303−54C>T, g.70336411G>A,* c.1392G>T, c.1923G>A, c.2052G>A, c.2823C>T, c.3135C>T, c.3843−12C>T, g.70666749G>A,* g.70644566G>A,* g.70331881G>A,* g.70319339C>T[133] g.70666635G>A,* g.70544853C>A,* g.70348949C>A,* g.70348913C>T,* g.70332914C>T,* g.70332890C>T,* g.70332272C>T,* g.70331795C>T,* g.70319359A>G*[134] g.70821018C>G g.70858273A>C[59]	—	c.76C>T, c.467A>G, c.942+19G>A, c.924+133G>C, c.1141+49G>A, c.1148−109C>T (associated with intellectual disability)[133]	Iossifov et al.[59] Peykov et al.[131] Homann et al.[132] Berkel et al.[133] Leblond et al.[134]

communication, altered motor coordination or other abnormal behaviour (Table 4).

Summary

These findings suggest that mutations in DLG genes might increase the risk of developing schizophrenia and autism-spectrum disorders, as well as related cognitive deficits, by contributing to the disruption of glutamatergic synapses. Although the neurobiological mechanisms underlying these disruptions are still unknown, some pathways can be inferred. For instance, mutations affecting *DLG2* might modify the tyrosine phosphorylation-based regulation of NMDA receptors, altering NMDA-receptor-related signalling. Similarly, mutations in *DLG4* might dysregulate NMDA receptor activity, because this protein also anchors different protein tyrosine kinases.[229] Other mechanisms might explain the association between *DLG4* and both schizophrenia and autism-spectrum disorders. It has been reported that DLG4 inhibits the interaction between dopamine receptor D1 and the NMDA receptor, preventing a reciprocal damaging overactivation of both receptors.[86] This suggests that reduced expression or dysfunction of this protein might dysregulate glutamatergic and dopaminergic homeostasis. Finally, since DLG4 enhances the expression of NMDA receptor subunits NR2A and NR2B[230] and the traffic of the NMDA receptor to synapses,[231] diminished expression or alterations in protein function might also compromise NMDA-receptor-mediated signalling transduction.

The DLGAP protein family

Some SNPs (Table 1) and SNVs (Table 2) in the *DLGAP1* gene and SNVs (Table 2) in the *DLGAP3* gene have been associated with schizophrenia. No studies have assessed the association between *DLGAP4* and schizophrenia or autism-spectrum disorders.

There is more evidence for an association between the *DLGAP2* gene and both disorders. The SNPs (Table 1), SNVs (Table 2) and CNVs (Table 3) in this gene have been associated with both schizophrenia and autism-spectrum disorders. Interestingly, some variants were coincident in both disorders (Table 1, Table 2 and Table 3).

On one hand, the SNP rs2906569 (A>G) in intron 1 and the missense SNP rs2301963 (C>A; P384Q) in exon 3 have been associated with both autism-spectrum disorders[115] and

Table 2: Single nucleotide variants in scaffolding genes associated with schizophrenia, autism-spectrum disorders and other clinical phenotypes of interest (part 3 of 3)

	Single nucelotide variants				
Gene	Schizophrenia	Autism-spectrum disorders	Schizophrenia and autism-spectrum disorders	Other associated phenotypes	Sources
SHANK3	g.49484091C>T g.49506476C>T[46,135] g.51117040G/A, g.51117200G/T, g.51117489C/T, g.51117580C/T, g.51117585G/A, g.51137217A/G, g.51143287C/T, g.51144513C/G, g.51153371G/A, g.51159735C/T, g.51159798A/G, g.51159802C/T, g.51159828G/A, g.51160154G/A, g.51169180A/G[51]	g.51117341C>G, g.51159953G>A, g.51169240A>G[136] g.51159293G>T*[129,136] c.670G>A[136,138] g.51121780C>T,* g.51159458G>T,* g.51113103C>T[136,140] g.51117094C>G,* g.51160615G>T[137] g.51142357C>T, g.51153464G>A,* g.51158686G>T,* g.51158945T>C,* g.51159965C>A,* g.51159988C>T,* g.51160086A>T,* g.51160057G>A[129] c.1527G>A[138] g.51113615T>C*[139] g.51121844A>G,* g.51123071C>T,* g.51159169G>T,* g.51160477C>G, g.51169213G>A,* g.51160589T>C[141] g.51159778G>A,* g.51160049C>T,*§ [141,142] c.1563G>A, c.1967G>A, c.4908C>T[142] g.1159884G>A,* g.51160018A>T, g.51169259C>T* (p.1572A>V), 51169364C>T,* 51169442G>A,* 51169459C>T, 51169463C>T,* 51169480G>A, g.51169207C>T* g.51169499G>A[143] c.612C>A, c.763C>T, c.898C>T, c.920C>G, c.1315C>T, c.1337G>T, c. 3761C>T, c.3764C>T, c.3836C>T, c.4025C>T, c.4405G>C, c.4406G>T, c.4490G>A, c.4720G>A[144] c.5008A>T[145]	g.49506159G>T[129,135,136]	g.49506476C>T, c.5008A>T, c.1527G>A (associated with intellectual disability)[46,138,145]	Awadalla et al.[46] Girard et al.[51] Leblond et al.[129] Gauthier et al.[135] Durand et al.[136] Boccuto et al.[137] Soorya et al.[138] Gauthier et al.[139] Durand et al.[140] Moessner et al.[141] Waga et al.[142] Schaaf et al.[143] Kelleher et al.[144] Cochoy et al.[145]
HOMER1	IVS4 þ[146]	c.195G>T, c.290C>T, c.425C>T, c.968G>A, c.1090C>T[144]			Kelleher et al.[144] Norton et al.[146]

*These variants have functional impact on the protein using the PolyPhen-2 or the Pmunt computer program.
†Chien and colleagues also found this variant in controls.[115]
‡Li and colleagues also found this variant in controls.[116]
§Waga and colleagues also found this variant in controls.[142]

schizophrenia.[116] Although the functional significance of rs2906569 is difficult to infer, it could affect either the final protein function or the regulation of gene expression by altering different processes, such as splicing, translation regulation or mRNA polyadenylation.[232] The missense variant rs2301963, in which CC homozygotes were overrepresented in patients with schizophrenia and patients with autism-spectrum disorders, could affect final protein activity according to bioinformatics analyses.[115]

On the other hand, 3 nonsynonymous exonic de novo variants (c.841C>G, c.2135C>T and c.2750C>T) have been identified in both schizophrenia[116] and autism spectrum disorders.[115] The c.841C>G and c.2750C>T mutations were predicted to damage protein function using PolyPhen-2 or Pmut.

Moreover, deletion of the chromosomic region 8p23.3 has been detected in patients with schizophrenia and patients with autism-spectrum disorders. This deletion and other CNVs spanning this gene have been found in patients with

Table 3: Copy number variants in scaffolding genes associated with schizophrenia, autism-spectrum disorders and other clinical phenotypes of interest (part 1 of 2)

	Copy number variants				
Gene	Schizophrenia	Autism-spectrum disorders	Schizophrenia and autism-spectrum disorders	Other associated phenotypes	Sources
DLG1	—	del:195971510_197675831[147]	del:3q29[45,50,57,148–156]	Deletion of 3q29 has been associated with intellectual disability,[148,149,154–156] developmental delay,[152] impaired social skills and repetitive behaviour[150,152]	Sanders et al.[45] Purcell et al.[50] Kirov et al.[57] Pinto et al.[147] Levinson et al.[148] Mulle et al.[149] Magri et al.[150] Szatkiewicz et al.[151] Quintero-Rivera et al.[152] Levy et al.[153] Willatt et al.[154] Ballif et al.[155] Sagar et al.[156]
DLG2	del:83472750_83842973 del:84006106_84226064[57] del:83680969_83943977[158]	del:84032216_84276593[157] dup:83108466_83378706[159]	—	—	Kirov et al.[57] Cuscó et al.[157] Walsh et al.[158] Egger et al.[159]
DLG4	—	dup:17p13.1_p13.2[153,160]	—	—	Levy et al.[153] Sanders et al.[160]
DLGAP2	dup:1436299_1642837[40]	dup:704383_1521910[60,147] del:1130900_6780950[162]	del:8p23.3[161,163–165]	Duplication of 1436299_1642837 has been associated with intellectual disability[40] Deletion of 8p23.3 has been associated with intellectual disability[161]	Guilmatre et al.[40] Pinto et al.[60] Pinto et al.[147] Chien et al.[161] Szatmari et al.[162] Costain et al.[163] Marshall et al.[164] Ozgen et al.[165]
SHANK1	—	del:55872189_55935995, del:55808307_55871709[130] del:19q13.33[166]	—	Deletion of 55872189_55935995 has been associated with impaired social communication skills, repetitive behaviours and higher functioning[130] Deletion of 55808307_55871709 has been associated with developmental abnormalities and disrupted social skills[130]	Sato et al.[130] Prasad et al.[166]
SHANK2	—	del:70154458_70220632[60] del:70077507_70506315, del:70119917_70187872, del: 70154458_70220632[147] del:70220882_70154208[133] dup:70520567_71017315[167] del:70332092(2)CTG > C[59]	—	Deletion of 70154458_70220632, 70077507_70506315 and 70119917_70187872 has been associated with language delay[60,147] Deletion of 70220882_70154208, 70077507_70506315 and 70119917_70187872 has been associated with intellectual disability[133,147]	Iossifov et al.[59] Pinto et al.[60] Berkel et al.[133] Pinto et al.[147] Gai et al.[167]

Table 3: Copy number variants in scaffolding genes associated with schizophrenia, autism-spectrum disorders and other clinical phenotypes of interest (part 2 of 2)

	Copy number variants				
Gene	Schizophrenia	Autism-spectrum disorders	Schizophrenia and autism-spectrum disorders	Other associated phenotypes	Sources
SHANK3	—	del:c.1320_1338, dup:c.1497+910bp/ins, del:c.1497+910bp/del[142] del:c.3259[168] del:48927548_51224208, del:48444959_51224208, del:49114430_51224208, del:44321641_51224208, del:46143471_51224208, del:44427703_51224208, del:46905533_51224208, del:49028732_51224208, del:49028732_51224208, del:43745129_51224208, del:50267252_51224208, del:45902119_51224208, del:42918711_51224208, del:45583935_51224208, del:48551989_51206201, del:51083118_51224208, del:45428606_51224208, del:44800014_51224208, del:44023173_51224208, del:43218614_51224208, del:46787434_51224208, del:49460840_51224208, del:51115526_51234443, del:45705241_51224208, del:49004395_51224208, del:42822943_51224208118, del:45159185_49582267[169] del:45159185_49582267, del:47996161_49512530, del:49468716_49485255, del:49470371_49567383, del:49470371_49480446[147]	del:22q13.3 [40,60,136,137,141,164,170–174]	Deletion of 22q13.3 has been associated with intellectual disability, language alteration,[60,137,139,170,174] developmental delay and impaired social interaction[140] Deletion of 45159185_49582267 has been associated with intellectual disability, hyperactivity, attention deficits and agressiveness[147] Deletion of 47996161_49512530 has been associated with severe intellectual disability and cortical atrophy[147] Deletion of 49470371_49567383 and 49470371_49480446 have been associated with language delay[147]	Guilmatre et al.[40] Pinto et al.[60] Durand et al.[136] Boccuto et al.[137] Gauthier et al.[139] Moessner et al.[141] Waga et al.[142] Pinto et al.[147] Marshall et al.[164] Nemirovsky et al.[168] Yuen et al.[169] Failla et al.[170] Crespi et al.[171] Sebat et al.[172] Wang et al.[173] Bonaglia et al.[174]
HOMER1	dup:78375511_78797532[167]	—	—	—	Gai et al.[167]

del = deletion; dup = duplication.

schizophrenia and patients with autism-spectrum disorders who have intellectual disability.[40,161]

To the best of our knowledge, there are no studies of the expression of *DLGAP2* in either autism-spectrum disorders or schizophrenia. Animal-model studies have suggested that alterations in this gene might lead to disadaptative social behaviour (Table 4).

Taken together, these findings suggest that disruptions of this gene might alter the function or expression of DLGAP2 and ultimately dysregulate its interplay with other PSD proteins, which could underlie the development of both schizophrenia and autism-spectrum disorders, as well as the manifestation of related clinical phenotypes, such as abnormal social behaviour. Interestingly, animal studies have found that DLGAP2 is vital for normal synaptic structure and function of the orbitofrontal cortex, a brain region that is implicated in the self-regulation of social-emotional behaviour.[233] There is also evidence that the orbitofrontal cortex is disrupted in patients with autism, and animal studies have indicated that a lesion of the orbitofrontal cortex may cause aggressive behaviour.[234]

The Shank protein family

Protein Shank1

Up to now, most of the SNVs in *SHANK1* have been associated with autism-spectrum disorders and not schizophrenia (Table 2). Deletions in *SHANK1* have also been associated with autism-spectrum disorders (Table 3), with some detected in patients with pronounced social dysfunction.

No expression studies have been carried out for either schizophrenia or autism-spectrum disorders, but animal-model studies seem to indicate that alterations in *SHANK1* could lead to impaired social skills (Table 4).

Protein Shank2

While SNVs in this gene have been identified in patients with schizophrenia and patients with autism-spectrum disorders

(Table 2), CNVs have been found only in people with autism-spectrum disorders, particularly with respect to intellectual disability or language delay (Table 3).

Among these mutations, the SNV (A1731S) detected in patients with schizophrenia is noteworthy. The authors of a study involving a functional assay in HEK293 cells reported that this variant has a significant effect on the F/G-actin ratio and concluded that diminished actin polymerization could lead to impairments in synapse formation and maintenance by reducing the presynaptic contacts.[131]

To the best of our knowledge, no expression studies have been performed for this gene. However, animal-model studies suggest that disruption of this gene could lead to the cognitive and social dysfunction associated with schizophrenia or autism-spectrum disorders by altering NMDA receptor function (Table 4).

Table 4: Expression, animal model and pharmacological studies on the reviewed scaffolding genes (part 1 of 2)

Gene	Expression studies	Functional studies	Sources
DLG1	Reduced expression of *DLG1* mRNA in PFC of schizophrenia patients[68] Reduced expression of *DLG1* protein in PFC of schizophrenia patients[175]	Administration of the NMDA receptor antagonist PCP caused an upregulation of *DLG1* gene transcription in the neocortex of rats[176]	Dracheva et al.[68] Toyooka et al.[175] Hiraoka et al.[176]
DLG2	Increased *DLG2* mRNA and decreased protein expression in prefrontal cortex and anterior cingulate cortex of schizophrenia patients[177]	PSD-93 mutant mice exhibited deficits in LTP[178] PSD-93 mutant mice showed cognitive abnormalities[179] PSD-93 mutant mice did not show any abnormality of synaptic structure or function in cerebellum[180]	Kristiansen et al.[177] Carlisle et al.[178] Nithianantharajah et al.[179] McGee et al.[180]
DLG3	Increased *DLG3* mRNA and protein expression in the thalamus of schizophrenia patients[181] Decreased *DLG3* protein expression in the thalamus of schizophrenia patients[182]	Mice lacking *DLG3* exhibited impairments of spatial learning[183]	Clinton et al.[181] Clinton et al.[182] Cuthbert et al.[183]
DLG4	Increased *DLG4* mRNA and decreased protein expression in ACC of schizophrenia patients[177] Increased *DLG4* mRNA and protein expression in thalamus of schizophrenia patients[182,185] Increased *DLG4* mRNA expression in the occipital cortex of schizophrenia patients[186] Decreased *DLG4* mRNA expression in the PFC of schizophrenia patients[69] Decreased *DLG4* mRNA and protein expression in the DLPFC of schizophrenia patients[187] Decreased *DLG4* protein expression in thalamus of schizophrenia patients[182] Decreased *DLG4* protein expression in hippocampus[188,189] Decreased mRNA expression in the striatum[190] No changes in either *DLG4* mRNA or protein expression in PFC of schizophrenia patients[175,186] No changes in either *DLG4* mRNA or protein expression in the hippocampus of schizophrenia patients[69,182]	*DLG4* mutant mice displayed schizophrenia and autism-spectrum disorder–like phenotypes[184] *DLG4* mutant mice displayed aberrant AMPA receptor-mediated transmission[178,191] *DLG4* mutant mice exhibited enhancement in LTP and deficit in LTD[178,192–194] *DLG4* mutant mice exhibited disrupted synaptic plasticity and impaired learning[192] Ketamine reduced *DLG4* mRNA in cortical regions of rats[195]	Ohnuma et al.[69] Toyooka et al.[175] Kristiansen et al.[177] Carlisle et al.[178] Clinton et al.[179] Clinton et al.[182] Feyder et al.[184] Clinton et al.[185] Dracheva et al.[186] Funk et al.[187] Toro et al.[188] Matosin et al.[189] Kristiansen et al.[190] Nakagawa et al.[191] Migaud et al.[192] Ehrlich et al.[193] Xu et al.[194] de Bartolomeis et al.[195]
DLGAP2	—	*DLGAP2* knockout mice displayed abnormal social behaviour[196]	Jiang-Xie et al.[196]
SHANK1	—	*SHANK1* mutant mice showed alterations in motor system and social behaviour[197–199] *SHANK1* mutant mice showed social communication deficits[200]	Silverman et al.[197] Hung et al.[198] Wöhr et al.[199] Sungur et al.[200]
SHANK2	—	*SHANK2*(–/ –) mutant mice were hyperactive and displayed autistim-like behaviours, including social interaction and repetitive jumping[201,202,203] *SHANK2*(–/ –) mutant mice exhibited fewer dendritic spines, areduced basal synaptic transmission and reduced frequency of miniature excitatory postsynaptic currents[201,203] *SHANK2*(–/ –) mutant mice showed a decrease in NMDA receptor function. Direct stimulation of the NMDA receptor with a partial agonist normalized its function and improved social interaction.[202]	Schmeisset et al.[201] Won et al.[202] Ha et al.[203]

Table 4: Expression, animal model and pharmacological studies on the reviewed scaffolding genes (part 2 of 2)

Gene	Expression studies	Functional studies	Sources
SHANK3	—	*SHANK3* mutant mice exhibited self-injurious repetitive grooming behaviours[204,206,207] and social interaction,[204,205,207,209,210] learning and memory[205] deficits. They also showed anxiety and motor deficits[206,208,209] *SHANK3* mutant mice showed deficits in glutamatergic transmission and synaptic plasticity and reduced synaptic concentrations of scaffolding proteins (e.g., DLGAP3, Homer1).[204,208–210] Re-expression of the *SHANK3* gene in adults led to improvements in synaptic protein composition, spine density and neural function, as well as selective rescue in autism-related phenotypes.[208] Insulin-like growth factor-1 reversed synaptic and behavioural deficits in *SHANK3* mutant mice[206] and phenotypic changes in human neuronal models of Rett syndrome.[211] *SHANK3B* knockout mice exhibited early hyperactivation and precocious maturation of corticostriatal circuits[212]	Arons et al.[204] Wang et al.[205] Bozdagi et al.[206] Peça et al.[207] Mei et al.[208] Bozdagi et al.[209] Yang et al.[210] Marchetto et al.[211] Peixoto et al.[212]
HOMER1	Increased Homer1a protein expression in hippocampal interneurons of schizophrenia patients[213] Increased Homer1a and decreased Homer1b protein expression in hippocampus of schizophrenia patients[189]	*HOMER1* knockout mice displayed impaired fear memory formation[214] and impaired LTP[215] *HOMER1* knockout mice showed abnormalities in motivational, emotional, cognitive and sensorimotor processing[216] *HOMER1* knockout mice also showed somatic growth retardation, poor motor coordination, enhanced sensory reactivity, learning deficits and increased aggression in social interaction[218] Overexpression of *HOMER1* in knockout mice reverted the cognitive and behavioural impairments[217] Exposure to novel environments upregulated *HOMER1* mRNA in the hippocampus of rats[219] Methamphetamine or cocaine administration upregulated *HOMER1* mRNA in the neocortex of rats[220] LSD or PCP administration upregulated *HOMER1* mRNA in the PFC of rats[221,222] 10Ketamine increased *HOMER1* mRNA in the cortical regions, striatum and nucleus accumbens of rats[195,223] Antipsychotics (haloperidol, olanzapine or clozapine) induced an increment of Homer1 protein expression in the cortex, the striatum, the caudate-putamen or nucleus accumbens of rats[107,224–228]	de Bartolomeis et al.[107] Matosin et al.[189] de Bartolomeis et al.[195] Leber et al.[213] Inoue et al.[214] Gerstein et al.[215] Szumlinski et al.[216] Lominac et al.[217] Jaubert et al.[218] Vazdarjanova et al.[219] Fujiyama et al.[220] Cochran et al.[221] Nichols et al.[222] Iasevoli et al.[223] Iasevoli et al.[224] Iasevoli et al.[225] Ambesi-Impiombato et al.[226] Polese et al.[227] Tomasetti et al.[228]

ACC = anterior cingulate cortex; AMPA = α-amino-3-hydroxy-5-methylisoxazole-4 propionic acid; LSD = lysergic acid; LTD = long-term depression; LTP = long-term potentiation; NMDA = *N*-methyl-D-aspartate; PCP = phencyclidine; PFC = prefrontal cortex.

Protein Shank3

There is accumulating evidence that *SHANK3* mutations contribute to the pathology of neurodevelopment disorders. Two SNVs in the *SHANK3* gene have been identified in patients with schizophrenia (Table 2), and different variants (SNPs, SNVs and CNVs) have been associated with autism-spectrum disorders (Table 1, Table 2 and Table 3). Among them, the missense variant G1011V (g.49506159G>T) located in exon 21 has been identified in patients with schizophrenia and patients with autism-spectrum disorders (Table 2). Further studies are needed to test whether this variant has any consequence for the protein function.

The R1117X nonsense mutation (g.49484091C>T) has been identified in exon 21 of the *SHANK3* gene in patients with schizophrenia and intellectual disability. This amino acid change resulted in a truncated form of the Shank3 protein that lacked the Homer-binding site, causing its loss of function.[46,135]

The A198G (g.51117341C>G) identified in people with autism-spectrum disorders generated a frameshift mutation that introduced a premature STOP codon at position 1227, leading to a truncated form of Shank3 that also caused its loss of function. This mutation disrupted actin polymerization, the regulation of spine formation and the molecular organization of the PSD.[136]

Two frameshift mutations causing premature STOP codons (g.51117094C>G and g.51160615G>T) resulted in the loss of protein function by losing the C-terminal region of the protein, which is crucial for interactions with other PSD proteins.[137]

Regarding CNVs, different studies have identified deletions in the *SHANK3* gene in patients with schizophrenia and patients with autism-spectrum disorders with intellectual

disability, developmental delay, language alterations or impaired social interaction (Table 3). Similarly, Phelan McDermid Syndrome (22q13.3 deletion syndrome), which includes deletion of the *SHANK3* gene, is characterized by neonatal hypotonia, global developmental delay, absence of speech, autistic behaviour and intellectual disability.[235]

Animal-model studies also appear to support a role for this gene in the cognitive and behavioural clinical phenotypes related to schizophrenia and autism-spectrum disorders. Shank3 mutant mice display reduced social interaction, affiliation behaviour, repetitive behaviour and communicative deficits (Table 4).

Summary

In summary, results for Shank proteins suggest that their disruption might underlie some of the cognitive and social dysfunction present in schizophrenia and autism-spectrum disorders. This is in line with the latest data showing that the prevalence of *SHANK3* mutations in people with autism-spectrum disorders is 0.5% to 0.7%, and that a *SHANK3* mutation is present in approximately 2% of people with both an autism-spectrum disorder and intellectual disability.[129,138,236] More specifically, from animal-model studies it has been suggested that these dysfunctions might be caused by alterations in the NMDA-receptor-related signalling pathway. There is evidence that *SHANK2* (−/−) mutant mice display abnormal NMDA receptor function and show alterations in behaviour and social skills.[201] Interestingly, when mutant mice were stimulated with NMDA receptor agonists, NMDA receptor function was normalized and their social interactions improved.[202] Regarding the pathophysiological mechanisms underlying the social deficits in *SHANK3* mutation, it has been reported that knockdown of the *SHANK3* gene in rat cortical cultures causes a loss of NMDA receptor function and alterations in its membrane trafficking through the disruption of the actin cytoskeleton.[237] Furthermore, Arons and colleagues showed that the loss of function of the *SHANK3* gene resulted in reduced glutamatergic synaptic transmission, whereas overexpression of this gene increased the number and size of excitatory synapses and the expression levels of other PSD proteins, such as DLG4 and Homer1.[204] Therefore, the behavioural and cognitive alterations present in patients carrying mutations in *SHANK* genes might be related to dysfunctions in NMDA-receptor-related glutamatergic signalling, which in turn might be caused by abnormalities in the interactions between Shank and other PSD proteins or anomalies in the actin polymerization processes.

The Homer protein family

Although 1 study reported a putative role for the *HOMER2* gene in schizophrenia (Table 1), it is principally the *HOMER1* gene that has been associated with schizophrenia and autism-spectrum disorders.

Three SNPs in *HOMER1* have been associated with schizophrenia (Table 1). Among them, rs4704560 has also been associated with the risk of developing psychotic symptoms in Parkinson disease (Table 1). In addition, 1 SNV and 1 CNV have been found in patients with schizophrenia (Table 2 and Table 3). As well, SNVs (Table 2) and CNVs (Table 3) have been detected in people with autism-spectrum disorders.

Expression studies have reported increases in Homer1a protein and decreases in Homer1b in the hippocampus of postmortem brain samples of schizophrenia (Table 4).

Animal-model studies suggest that *HOMER1* transcripts might control cognitive and behaviour functions (Table 4). It has also been suggested that mutations in *HOMER1* might increase the risk of developing schizophrenia by dysregulating NMDA receptors and their associated signalling pathway. Pharmacological studies have shown that the NMDR inhibitor phencyclidine (PCP) and the NMDA receptor antagonist ketamine increase *HOMER1* mRNA in rat prefrontal cortex and ventral striatum and nucleus accumbens, respectively. Other studies show that *HOMER1* mRNA and/or related protein expression levels are modified by psychotomimetic drugs and the antipsychotic haloperidol (Table 3).

Summary

Overall, although *HOMER1* has been associated with both schizophrenia and autism-spectrum disorders, genetic, expression and animal model studies do not provide conclusive results. This could be because different Homer1 isoforms have different functions. It has been suggested that the long isoform Homer1c is implicated in the regulation of working memory and sensorimotor function, whereas Homer1a could modulate the behavioural and emotional area.[217] Additionally, some studies report that the balance between long and short Homer forms determines the normal functioning of the synaptic architecture and function and influences synaptic plasticity dynamics[238]; therefore, an alteration of this balance could dysregulate synaptic signalling, leading to neurochemical, structural and behavioural changes.[217,218]

Discussion

Accumulating evidence supporting biological overlap between schizophrenia and autism-spectrum disorders has fuelled research into common underlying mechanisms to provide a better understanding of the etiology of these disorders, their diagnosis and treatment. One such mechanism involves the synaptic plasticity in which the PSD structure plays a key role. This review has summarized genetic variants in the main scaffolding genes of the PSD that have been associated with schizophrenia and/or autism-spectrum disorders to date. Moreover, evidence coming from genetic, brain expression and animal model studies suggests that genetic variants in scaffolding genes could contribute to the deregulation of the glutamate receptor signalling pathways of the PSD, which may be involved in the pathophysiology of schizophrenia and autism-spectrum disorders, and the development of related shared phenotypes, such as cognitive or social dysfunction.

Such a cross-disorder effect of scaffolding gene and protein dysregulation seems consistent with their role as a dynamic complex that regulates cell signalling pathways and determines the specificity of information flow in intracellular networks.[72] Because scaffolding proteins coordinate the excitatory

synaptic transmission and mediate functional changes at the synapse, thus regulating synaptic plasticity among other processes,[73] they can be seen as crucial pieces of the complex puzzle of synaptic homeostasis maintenance. The fact that common (SNPs) and rare (SNVs and CNVs) variants have been identified in scaffolding genes in both schizophrenia and autism-spectrum disorders is in agreement with the view that both kinds of variants complementarily and heterogeneously underlie the shared genetic susceptibility to these disorders (Table 5) by generating synaptic instability.

From the present review, it is possible to infer that patients with schizophrenia or autism-spectrum disorders primarily share CNVs that include the complete length of one or more genes. In this regard, CNVs in the PSD genes seem to increase the risk of developing either schizophrenia or autism-spectrum disorders. For instance, deletions of the chromosomal region 3q29, which includes the *DLG1* gene, have been related to both schizophrenia and autism-spectrum disorders.[45,49] Taking into account the usually large effect size of rare variants on a phenotype, we should not be surprised that alterations in the number of copies (deletions or duplications) of these genes have an impact on PSD functioning and plasticity. In addition to being associated with specific disorders, these variants have been associated with certain phenotypes in these or other disorders, or even in healthy controls. Pocklington and colleagues showed that rare CNV burden may be relevant to cognitive dysfunction in patients with schizophrenia,[239] and Stefansson and colleagues found that CNVs conferring risk of either schizophrenia or autism-spectrum disorders, including CNVs in the *DLG1* and *DLG2* genes, also affect cognitive function in healthy controls.[240] Other studies have similarly detected that mutations in PSD genes, including some of the scaffolding genes reviewed here, such as *DLG3*[83] or *SHANK3*,[241] are present in patients with intellectual disability.

This review has also provided evidence that, although several SNPs and SNVs in the scaffolding genes have been associated with schizophrenia or autism-spectrum disorders, only a few have been reported in both: 2 SNPs and 3 SNVs in the *DLGAP2* gene and 1 SNV in the *SHANK3* gene (Table 5). Variants that occur in both diagnoses might be targets of special interest for our understanding of common pathophysiological mechanisms and shared clinical features. Although it is difficult to infer the functional significance of these variants, bioinformatic analyses have indicated that some of the *DLGAP2* gene variants (rs2301963, c.841C>G and c.2750C>T) might affect final protein function or expression. In relation to *SHANK3*, to our knowledge, there is no available information about the functionality of the missense SNV (G1011V) that has also been found associated with both disorders.

Nevertheless, the general lack of specificity observed here can be explained in terms of the pleiotropic nature of scaffolding genes. Variants in different scaffolding genes, either at the allelic or the gene level, may dysregulate the homeostasis of the PSD, which is finally expressed as features associated with different neurodevelopment disorders. In addition

Table 5: Summary of variants in scaffolding genes associated with both schizophrenia and autism-spectrum disorders

Genes	SNPs	SNVs	CNVs	Sources
DLG1	—	—	del:3q29[45,50,57,148–156]	Sanders et al.[45] Purcell et al.[50] Kirov et al.[57] Levinson et al.[148] Mulle et al.[149] Magri et al.[150] Szatkiewicz et al.[151] Quintero-Rivera et al.[152] Levy et al.[153] Willatt et al.[154] Ballif et al.[155] Sagar et al.[156]
DLGAP2	rs2906569, rs2301963[115,116]	c.841C>G, c.2135C>T c.2750C>T[115,116]	del:8p23.3[161,163–165]	Chien et al.[115] Li et al.[116] Chien et al.[161] Costain et al.[163] Marshall et al.[164] Ozgen et al.[165]
SHANK3	—	g.49506159G>T[129, 135,136]	del:22q13.3[40,60,136,137,141,164,170–174]	Guilmatre et al.[40] Pinto et al.[60] Leblond et al.[129] Gauthier et al.[135] Durand et al.[136] Boccuto et al.[137] Moessner et al.[141] Marshall et al.[164] Failla et al.[170] Crespi et al.[171] Sebat et al.[172] Wang et al.[173] Bonaglia et al.[174]

CNV = copy number variant; del = deletion; SNP = single nucleotide polymorphism; SNV = single nucleotide variant.

to this pleiotropy, the polygenic nature of psychiatric disorders and the polygenic nature of the intermediate molecular pathways known to underlie at least part of the autism-spectrum disorders/schizophrenia pathology (such as the proper functioning of the PSD) should also be considered. This directly links with additional genetic phenomena, such as gene–gene interactions. In recent years, the gene-pathways methodology has been developed to study whether different genes with similar functions are jointly associated with a single phenotype. So far, only a few studies have assessed the effect of common variance in scaffolding genes as a functional gene set or the epistatic effects of other related PSD functional gene sets on the risk of schizophrenia or autism-spectrum disorders. One recent study explored the enrichment of schizophrenia-associated ultra-rare variants and found a significant enrichment of disrupting ultra-rare variants among genes defined as encoding interactors with DLG4 and ARC and NMDA receptors.[70] Another study observed an enrichment of SNPs associated with autism-spectrum disorders in gene sets related to synaptic structure and function, including genes related to scaffolding proteins, β-catenin nuclear pathways, glutamate receptor activity and adherents junctions.[67] In addition, although not significant after correction, a nominal association between a PSD protein defined gene set (including ARC and NMDA receptor complexes) with schizophrenia has been reported.[36] In all, the effect of common and rare variants in scaffolding genes on schizophrenia and autism-spectrum disorders reflects the complex and heterogeneous genetic architecture of these disorders, and further analyses of gene sets could facilitate the untangling of this complexity.

In addition to genetic data, expression and animal-model studies have indicated the importance of scaffolding genes in schizophrenia and autism-spectrum disorders. There is evidence that patients with schizophrenia or autism-spectrum disorders display deviations from normal scaffolding protein brain expression levels, supporting the hypothesis that the deregulation of these genes might underlie the neurobiology of both disorders. However, to our knowledge, there are no brain expression studies of the 2 genes (*DLGAP2* and *SHANK3*) in which overlapping variants that predispose individuals to schizophrenia and/or autism-spectrum disorders were found.[52,115,116,135,242] Further research is required to test whether these coincident genetic variants contribute to modifying protein expression levels. In contrast, studies with animal models have shown the importance of scaffolding genes in ensuring cognitive and social function. We have reviewed different studies in which mice with scaffolding protein mutations show schizophrenia and autistim-like phenotypes.[184,205] The use of animal models is extremely useful for understanding how changes in the gene sequence can affect phenotypes. As an example of the potential importance of animal models, there are *SHANK3*-deficient mice in which synaptic deficits were reversed with insulin-like growth factor-1.[206] In a recent pilot study, insulin-like growth factor-1 has been used to treat 9 children with autism, and preliminary results have shown a reduction in social deficits.[243]

Limitations

Some limitations of this review should be acknowledged. First, since the reviewed association studies do not always include the same genetic regions or variants, coincident variants can be linked to shared genetic variability between schizophrenia and autism-spectrum disorders, but noncoincidence cannot be interpreted as a lack of it. Second, patients with low IQ scores are generally excluded from association studies. Since the scaffolding genes reviewed here seem to contribute to cognitive phenotypes, it is plausible to hypothesize that the effects of SNPs and SNVs in these genes were detected less often than they actually occur. Third, the relationship between the effect size associated with common and rare variants, the statistical power needed to detect these effects and the sample sizes in the studies reported in the different articles reviewed should be considered. The odds ratios associated with schizophrenia risk SNPs are typically about 1.10 to 1.50, whereas schizophrenia-associated CNVs confer a significantly increased risk of illness (odds ratios for several CNVs exceed 8).[244] Therefore, rare variants can more easily create significant genome-wide associations than common variants.[245]

Conclusion

Advances in genetic technologies, together with the assembly of large patient cohorts, have made it possible to identify some genes and biological pathways involved in both schizophrenia and autism-spectrum disorders. Among them, scaffolding genes implicated in the PSD have been repeatedly associated with schizophrenia and autism-spectrum disorders, pointing toward these genes' common involvement in the neurobiology of these disorders and in some shared clinical phenotypes, such as cognitive and social impairment. This review summarizes evidence that many different variants could introduce numerous slight alterations in the PSD pathway, leading to its inappropriate development or insufficiently robust response to environmental insults.

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Supervisor's report on the contribution of the PhD applicant to the article

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as the supervisors of the present doctoral thesis by Jordi Soler Garcia, hereby certify that the participation of the PhD applicant in the article *"Genetic variability in scaffolding proteins and risk for schizophrenia and autism-spectrum disorders: a systematic review"* included the following tasks:

- Conception and design of the study
- Literature review
- Writing of the first manuscript draft
- Critical revision of the article for intellectual content

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Barcelona, May 14th 2021

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ZNF804A gene and cannabis use: interaction on the risk for psychosis in a non-clinical sample

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The interaction between the ZNF804A gene and cannabis use on the risk of psychosis in a non-clinical sample

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ABSTRACT

The *ZNF804A* gene and cannabis use are risk factors for psychosis and both have also been associated with schizotypal traits. This study aimed to investigate: i) the association of lifetime cannabis use (and its dose effect) with schizotypal personality traits, and ii) whether the genetic variability at *ZNF804A* gene modulates that association.

Our sample consisted of 385 Spanish non-clinical subjects (43.1% males, mean age = 21.11(2.19)). Schizotypy was evaluated using the three factors of the Schizotypal Personality Questionnaire-Brief (SPQ-B): Cognitive-Perceptual (SPQ-CP), Interpersonal (SPQ-I) and Disorganized (SPQ-D). Subjects were classified according to their frequency of cannabis consumption, and dichotomized as users or non-users. The effects of a genetic variant of *ZNF804A* (rs1344706) and cannabis use, as well as their interaction, on each of the three SPQ-B factors were assessed using linear models and permutation tests. Sex, SCL anxiety scores and use of other drugs were included as covariates.

Our analysis showed a significant relationship between *ZNF804A* and SPQ-I: AA genotype was associated with higher scores ($\beta = 0.885$ $p_{FDR} = .018$). An interaction between the AA genotype and lifetime cannabis use was found in SPQ-CP ($\beta = 1.297$ $p_{FDR} = 0.018$). This interaction showed a dose–effect pattern among AA subjects: schizotypy scores increased with increasing frequency of cannabis use (sporadic users: $\beta = 0.746$ $p_{FDR} = 0.208$; monthly users: $\beta = 1.688$ $p_{FDR} = 0.091$; intense users: $\beta = 1.623$ $p_{FDR} = 0.038$).

These results add evidence on that the *ZNF804A* gene is associated with schizotypy and suggest that the interaction between cannabis use and *ZNF804A* genotype could modulate psychosis proneness.

1. Introduction

Psychotic disorders, including schizophrenia, are among the most severe and impairing medical conditions, with lifetime prevalence around 3% (Perala et al., 2007). They are multifactorial disorders determined by genetic and environmental risk factors, as well as their interaction (European Network of National Networks studying Gene-Environment Interactions in Schizophrenia (EU-GEI), 2014).

Regarding genetic factors, twin and family studies have estimated that the heritability of schizophrenia is between 64% and 81% (Lichtenstein et al., 2009; Sullivan et al., 2003). Moreover, genome-wide association studies have reported a substantial polygenic component that contributes to the risk for schizophrenia (Gratten et al., 2014; Purcell et al., 2009) and have allowed several candidate genes to be identified. One of the most relevant is the *ZNF804A* gene, with many studies enhancing our understanding of its involvement in

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schizophrenia (e.g. Gratten et al., 2014; Hess and Glatt, 2014; Riley et al., 2010; Ripke et al., 2014; Steinberg et al., 2011; Williams et al., 2011).

ZNF804A (2q32.1) is expressed throughout the fetal and adult human brain (Hill and Bray, 2012; Tao et al., 2014). Despite the exact functions of *ZNF804A* still remaining unclear, proteins with zinc finger domains are known to play a variety of roles, including the regulation of gene expression and DNA–protein interactions (Brayer and Segal, 2008; Hess and Glatt, 2014; Laity et al., 2001). In this regard, molecular and bioinformatics studies suggest that *ZNF804A* probably plays pivotal roles in cell physiology, neurodevelopment regulation (Riley et al., 2010) and synaptic plasticity (Deans et al., 2016; Hess and Glatt, 2014; Hill and Bray, 2012).

As a putative transcription factor, *ZNF804A* has a large number of potential targets (Hill et al., 2012). Interestingly, some of the genes regulated by *ZNF804A*, such as the Dopamine Receptor D2 (*DRD2*) or Catechol-O-Methyltransferase (*COMT*), are directly involved in dopaminergic transmission and have been associated with schizophrenia (Girgenti et al., 2012). Therefore, current evidence suggests that dysregulation of ZNF804A could contribute to the altered neuronal and synaptic structures that are associated with psychotic disorders (Penzes et al., 2011).

Within the *ZNF804A* gene, the rs1344706 single nucleotide polymorphism (SNP) has repeatedly been associated with psychosis (O'Donovan et al., 2008; Purcell et al., 2009; Ripke et al., 2014; Steinberg et al., 2011; Williams et al., 2011); with the A allele identified as the variant associated with increased risk. In addition, two independent studies have shown that the risk allele of rs1344706 is associated with reduced expression of *ZNF804A* RNA, both in fetal brain tissue and in individuals with schizophrenia, bipolar disorder or major depressive disorder (Hill and Bray, 2012; Tao et al., 2014).

Two studies have also associated rs1344706 with vulnerability to psychosis, as indicated by determining the schizotypy of individuals (Stefanis et al., 2013; Yasuda et al., 2011). Schizotypy involves a set of personality traits encompassing behavior, cognition and emotions, that resemble the signs and symptoms of psychotic disorders in the general population (Raine, 2006). These similarities are suggested to reflect the existence of overlapping etiological factors between schizotypy and psychotic symptoms (Fanous et al., 2007). Therefore the study of the genetic underpinnings of vulnerability traits in non-clinical samples constitutes a useful framework within which to study etiological factors of psychotic disorders (Barrantes-Vidal et al., 2015).

Cannabis use is one of the environmental risk factors most strongly implicated in the emergence of psychotic symptoms and disorders (Minozzi et al., 2010; Moore et al., 2007). In addition, a meta-analysis has shown evidence of the existence of a dose–response relationship between the intensity of cannabis use and the risk for psychosis (Marconi et al., 2016).

Cannabis induces psychotic symptoms through the activation of the endocannabinoid system, which is an endogenous system that modulates dopamine neurotransmission (Covey et al., 2017). Interestingly, cannabis use has been associated with the positive and the disorganized dimensions of schizotypy (Cohen et al., 2011; Fridberg et al., 2011; Schubart et al., 2011; Szoke et al., 2014). However, only a relatively small proportion of cannabis users develop psychotic symptoms, which means that other factors might explain the connection between cannabis use and psychosis risk (Decoster et al., 2012). In this context, the fact that familial correlation of schizotypal scores varies depending on exposure to cannabis, confirms the importance of gene–cannabis interactions in the expression of psychosis vulnerability markers (Kahn et al., 2011; van Winkel and GROUP Investigators, 2015). Some studies have already shown the effect of the interaction between genes related to dopaminergic neurotransmission (*COMT* and *AKT1*) and cannabis use on the risk for psychosis (Radhakrishnan et al., 2014).

Considering all this, analysis of the interaction between *ZNF804A* and cannabis use is of interest in order to increase our knowledge of the mechanisms underlying the relationship between cannabis use and risk of psychosis. Therefore, we studied: i) the association of lifetime cannabis use (and its dose effect) with schizotypal personality traits in a non-clinical sample, ii) whether genetic variability in the *ZNF804A* gene modulates that association.

2. Methods

2.1. Sample

Our sample consisted of 385 subjects from the general Spanish population who were recruited in 2004–05 at the campus of the Universitat Jaume I in Castelló (Spain). Exclusion criteria were the presence of any major medical illness affecting brain function, neurological conditions, and a personal history of head injury or psychiatric medical treatment. These were screened for by trained psychologists using a short interview designed for this study that included selected items of psychiatric diagnosis structured scales such as the Structured Clinical Interview for DSM-IV (SCID-I) (First et al., 1999) and the Familiar Interview for Genetic Studies (FIGS) (Maxwell, 1992). The participants were asked specific questions on psychiatric treatment, psychotropic medication, hospital admissions and suicide attempts.

Ethical approval was obtained from local research ethics committees. All the participants provided written consent after being informed of the study procedures and implications. All the procedures were carried out in accordance with the Declaration of Helsinki.

2.2. Measures

Schizotypal personality was measured using the Schizotypal Personality Questionnaire-Brief (SPQ-B) (Raine and Benishay, 1995). The SPQ-B consists of a 22-item self-report scale and is comprised of three separate factors (cognitive-perceptual (SPQ-CP), interpersonal (SPQ-I) and disorganized (SPQ-D)). The SPQ-CP and the SPQ-I factors included 8 items each, and the SPQ-D, 6; and the final score for each factor is calculated as the sum of all the items in each factor. These factors include the evaluation of the presence of odd ideas, paranoia, a lack of close personal relationships, suspiciousness and odd behavior and speech. Since the relationship between cannabis use and schizotypy is suggested to be influenced by other psychopathological traits, such as high levels of anxiety (Braunstein-Bercovitz et al., 2000; Reilly et al., 1998), the participants were tested for their anxious symptomatology by means of the 23-item scale of the revised version of the Symptom Check List (SCL-90-R) (Derogatis and Melisaratos, 1983). Clinical information was available for all individuals.

Lifetime cannabis use was assessed using one question regarding the frequency of consumption: “never”, “sporadic”, “monthly” or “intense” (weekly or daily)”. This variable was then dichotomized into: cannabis user (intense and monthly) or cannabis non-user (sporadic and never). Similarly, the participants were asked about the use of other illicit drugs (i.e. amphetamine-type stimulants, cocaine, heroin and other opioids, or ecstasy) and were classified as other drug users (daily, weekly, and monthly) or other drug non-users (sporadic or never). Cannabis use information was available for all individuals and data on other drugs for 98.7%.

2.3. Molecular analysis

Genomic DNA from each individual was extracted from buccal mucosa samples by means of a cotton swab using the Real Extraction DNA Kit (Durviz S.L.U., Valencia, Spain). The SNP rs1344706 in the *ZNF804A* gene was determined using Applied Biosystems TaqMan 5′ exonuclease assays. PCR plates were read on an ABI PISM 7900HT instrument using SDS 2.4 software (Applied Biosystems). The genotyping call rate was 100%.

2.4. Statistical analysis

All data were processed using Stata v.14 (StataCorp, 2013) and R (R Core Team, 2014).

Student's *t*-test and ANOVA were used to compare the means of continuous variables between two or more groups, respectively. A chi-squared test was performed to analyze the distribution of qualitative variables between groups and also to examine the Hardy-Weinberg equilibrium.

The effect of genetic and environment factors and their interaction on each of the three SPQ-B factors (SPQ-CP, SPQ-I and SPQ-D) was tested by means of linear models. The models included the main effect of the independent variables (rs1344706 genotypes and cannabis use) and their interaction (rs1344706*cannabis use). Sex, SCL anxiety scores and the use of other drugs were included as covariates. Cannabis non-use and the CC genotype were taken as reference categories. The models were first tested with lifetime cannabis use as a dichotomized variable. To gain a better understanding of the significant effect of the interaction, the relationship of the genotype and SPQ-CP scores was tested separately in cannabis non-users and users by means of linear regression. In addition, to test whether there is a cannabis dose effect, the same analysis was performed including the frequency of cannabis use instead of the binary value.

Due to the non-normal distribution of SPQ-B scores, in all linear models permutation tests were used to quantify the statistical significance of the variables (permuco package in R; https://cran.r-project.org/web/packages/permuco/index.html). Permutation tests are non-parametric tests since they do not rely on assumptions about the distribution of the data. For example, these methods have been successfully applied to compare conditions in experimental design using functional magnetic resonance imaging (fMRI) (Winkler et al., 2014). Results from permutation tests were corrected for multiple comparisons using the false discovery rate (FDR) correction (Benjamini and Hochberg, 1995). FDR-adjusted p-values < .05 were considered statistically significant.

3. Results

3.1. Sample description

Participants (n = 385) were university students (43.1% males) with a mean age at interview of 21.11 years (sd = 2.19). The SPQ-B and SCL-anxiety mean scores (sd) were as follows: SPQ-CP 1.57 (1.49), SPQ-I 2.76 (2.12), SPQ-D 1.15 (1.23) and SCL-A 4.20 (4.99). SPQ-B factor scores in relation sex, genotype and cannabis use are given in Table 1.

A total of 71.43% of individuals were classified as cannabis non-users (49.81% never, 50.19% sporadic) and 28.57% as cannabis users (38.18% monthly, 61.82% intense); while 4.43% of individuals were other drug users.

The genotype frequencies were in Hardy-Weinberg equilibrium. They were as follows: 31.2% AA, 47.0% AC and 21.8% CC. The minor allele frequency (MAF) of the sample (45.3%) was similar to that reported for the European population in the 1000 Genomes Project (MAF = 38.4%).

The effect of the covariates (sex, SCL anxiety scores and the use of other drugs) on SPQ mean scores was analyzed. Significant differences between women and men in SPQ-I ($t = -2.71$ $p = .007$) and SPQ-D ($t = -4.13$ $p < .001$) were observed. Anxiety scores significantly correlated with the three schizotypy factors ($p < .001$).

When cannabis non-users were compared to cannabis users, no statistically significant differences in age, genotype, SCL scores or SPQ-CP and SPQ-I factors were observed. However, there were significant differences in sex (60.7% of cannabis non-users and 47.3% of cannabis users were women ($\chi^2 = 5.83$ $p = .016$)) and SPQ-D (cannabis users had higher SPQ-D scores than non-users ($t = -2.38$ $p = .017$)).

No effect of the genotype on lifetime cannabis use was observed.

Table 1
Mean scores of the three schizotypy factors, according to sex, *ZNF804A* genotype (rs1344706) and cannabis use variables.

		SPQ-CP	SPQ-I	SPQ-D
Sex[a]	Female	1.53 (1.51)	2.51 (2.09)	0.94 (1.08)
	(n = 219)	[1.33–1.73]	[2.23–2.78]	[0.90–1.08]
	Male	1.61 (1.47)	3.09 (2.11)	1.43 (1.36)
	(n = 166)	[1.39–1.84]	[2.77–3.41]	[1.23–1.64]
Genotype[b]	AA	1.47 (1.48)	3.19 (2.15)	1.21 (1.32)
	(n = 120)	[1.21–1.74]	[2.81–3.58]	[0.97–1.44]
	AC	1.69 (1.51)	2.65 (2.08)	1.21 (1.23)
	(n = 181)	[1.47–1.90]	[2.35–2.95]	[1.03–1.39]
	CC	1.45 (1.45)	2.36 (2.08)	0.96 (1.10)
	(n = 84)	[1.14–1.76]	[1.92–2.81]	[0.72–1.20]
Cannabis (dichotomized)[b]	Non-users	1.57 (1.48)	2.72 (2.12)	1.06 (1.20)
	(n = 275)	[1.39–1.74]	[2.46–2.97]	[0.92–1.20]
	Users	1.61 (1.51)	2.91 (2.12)	1.4 (1.31)
	(n = 110)	[1.33–1.90]	[2.51–3.31]	[1.15–1.64]
Cannabis (frequency)[b]	Never use	1.42 (1.34)	2.82 (2.24)	0.93 (1.13)
	(n = 137)	[1.19–1.64]	[2.44–3.20]	[0.74–1.12]
	Sporadic	1.71 (1.60)	2.61 (2.00)	1.19 (1.26)
	(n = 138)	[1.44–1.98]	[2.28–2.95]	[0.98–1.40]
	Monthly	1.47 (1.53)	3.02 (2.30)	1.5 (1.61)
	(n = 42)	[1.01–1.94]	[2.32–3.72]	[1.01–1.98]
	Intense	1.70 (1.50)	2.85 (2.02)	1.33 (1.10)
	(n = 68)	[1.34–2.06]	[2.37–3.73]	[1.07–1.60]

Schizotypy factors from SPQ-B: CP (Cognitive-Perceptual), I (Interpersonal), D (Disorganized).

[a] Significant differences between females and males on SPQ-I ($t = -2.71$ $p = .007$) and SPQ-D ($t = -4.13$ $p < .001$) were observed.

[b] Effect of genotype and cannabis use on SPQ-B factors are given in Table 2.

3.2. Effects of lifetime cannabis use and ZNF804A genotype on SPQ-B

We first analyzed the dichotomized cannabis use variable. In relation to the SPQ-I factor, the genotype AA was significantly associated with higher scores ($\beta = 0.885$ $p_{FDR} = 0.018$). The mean (sd) SPQ-I scores for each genotype were as follows: AA (3.19 (0.19)), AC (2.65 (0.15)) and CC (2.36 (0.22)). No effect of lifetime cannabis use or the genotype x cannabis interaction was detected on SPQ-I.

No effect of genotype or cannabis was observed on SPQ-CP, while the interaction analysis showed the interplay between the genotype AA and lifetime cannabis use ($\beta = 1.297$ $p_{FDR} = 0.018$) (Table 2A). Specifically, when the effect of the genotype on SPQ-CP scores was tested separately in cannabis users and non-users, a significant effect was observed only in cannabis users, with AA homozygotes showing the highest scores (Fig. 1).

This interaction was reevaluated taking into account the frequency of cannabis use and we observed a cannabis dose effect among AA subjects on SPQ-CP scores: a significant interaction effect was specifically detected in intense users (sporadic users: $\beta = 0.746$ $p_{FDR} = 0.208$; monthly users: $\beta = 1.688$ $p_{FDR} = 0.091$; intense users: $\beta = 1.623$ $p_{FDR} = 0.038$) (Table 2B).

Finally, no significant effect of the genotype, lifetime cannabis use or their interaction was observed on SPQ-D.

4. Discussion

This study provides evidence that the *ZNF804A* gene has a certain effect on psychosis risk, measured as schizotypal traits in healthy subjects. In addition, our findings suggest, for the first time, that genetic variation in the *ZNF804A* gene may modulate the relationship between the lifetime cannabis use and psychotic proneness.

On the one hand, we observed an association of the polymorphism rs1344706 with SPQ-I. Specifically, we found that AA homozygotes showed the highest Interpersonal schizotypal scores. This result is in line with different studies that report an association of the A allele with schizophrenia risk (Purcell et al., 2009; Williams et al., 2011) and also

Table 2
Results of the analysis of the effect of Cannabis use, *ZNF804A* genotype (rs1344706) and their interaction on SPQ-CP. Three covariates were included (age, sex, SCL-R anxiety scores). Permutation tests *p*-values and corrected (FDR) p-values are given. Part A shows the results of the analysis with the cannabis variable dichotomized. Part B shows the results of the analyses with the frequency of cannabis use.

	β	SE	Permutation p-value	FDR p-value
A. SPQ cognitive-perceptual factor (R^2 = 0.179)				
Cannabis use	−0.651	0.34	0.064	0.171
ZNF804A (AC)	0.165	0.211	0.439	0.439
ZNF804A (AA)	−0.229	0.224	0.312	0.439
Cannabis use * *ZNF804A* (AC)	0.557	0.398	0.170	0.341
Cannabis use * *ZNF804A* (AA)	1.297	0.452	**0.005**	**0.018**
Sex	*0.115*	*0.141*	*0.419*	*0.706*
Anxiety	*0.122*	*0.013*	*0.0001*	*0.0008*
Other drugs	*0.111*	*0.136*	*0.409*	*0.439*
B. SPQ cognitive-perceptual factor (R^2 = 0.174)				
Cannabis (sporadic)	−0.360	0.351	0.310	0.395
Cannabis (monthly)	−0.792	0.510	0.119	0.208
Cannabis (intense)	−0.780	0.459	0.087	0.204
ZNF804A (AC)	−0.078	0.290	0.790	0.790
ZNF804A (AA)	−0.579	0.308	0.064	0.204
Cannabis (sporadic) * *ZNF804A* (AC)	0.534	0.428	0.211	0.329
Cannabis (monthly) * *ZNF804A* (AC)	0.638	0.610	0.299	0.395
Cannabis (intense) * *ZNF804A* (AC)	0.903	0.529	0.087	0.204
Cannabis (sporadic) * *ZNF804A* (AA)	0.746	0.450	0.105	0.208
Cannabis (monthly) * *ZNF804A* (AA)	1.688	0.714	**0.020**	0.091
Cannabis (intense) * *ZNF804A* (AA)	1.623	0.588	**0.005**	**0.038**
Sex	*0.111*	*0.143*	*0.445*	*0.520*
Anxiety	*0.122*	*0.014*	*0.0001*	*0.001*
Other drugs	*0.070*	*0.145*	*0.628*	*0.676*

Significant GxE interactions are highlighted in bold.

with worse clinical outcomes (O'Donovan et al., 2008; Wickramasinghe et al., 2015). It is also consistent with two previous studies that found this variant to be associated with schizotypy as evaluated using the SPQ (Stefanis et al., 2013; Yasuda et al., 2011). In a non-clinical population, Yasuda et al. (2011) reported that A carriers showed higher disorganized schizotypy; while not observing differences in the other factors. Meanwhile, Stefanis et al. (2013) reported an association between the paranoid and disorganized factors and the rs1344706 polymorphism in another non-clinical population, although the direction of the association was not as expected. Differences between those studies and ours might be explained by sample characteristics, such as differences in sample size, ethnic origin or age and sex distributions. For instance, the 2011 study consisted of 176 Japanese individuals with a mean age of 36.8 (sd = 11.5) years. In the 2013 study, although the subject's mean age (21 years, sd = 1.9) was similar to that in our study, all the subjects were men in military service. Despite these differences, our results add on the body of evidence that the *ZNF804A* gene plays a role in schizophrenia proneness. Another recent study also indicated such a relationship, based on another vulnerability measure: Psychotic-Like Experiences (de Castro-Catala et al., 2017).

On the other hand, GxE interaction analyses have shown that lifetime cannabis seems to act as a modifier of the association between the rs1344706 genotype and cognitive–perceptual schizotypy. Among cannabis users, AA individuals showed the highest SPQ-CP scores. In addition, we also observed that the interaction effect followed a dose–response relation within AA subjects: SPQ-CP scores increased as frequency of cannabis use increased.

This suggests that the *ZNF804A* gene modifies sensitivity to cannabis; specifically, the AA genotype is associated with higher psychosis proneness among intense cannabis users. As mentioned, a dose–response relationship between the frequency of cannabis use and the risk for psychosis has been repeatedly reported and confirmed by a meta-analysis (Marconi et al., 2016). Therefore, our data indicate that this relationship could be mediated by the genetic background. Although no previous studies have assessed the interaction between *ZNF804A* and cannabis use frequency, van Winkel et al. (2011) noted a significant interaction between frequency of cannabis use and the *AKT1* gene.

In our models, the interaction between *ZNF804A* and cannabis use we identified explains part of the schizotypy variance (R^2 = 0.17). Other mechanisms have to be considered to fully understand the relationship between *ZNF804A*, cannabis use and psychotic proneness. For instance, it is well known that there is a genetic background for the likelihood of using cannabis (gene-environment correlation) (Kendler et al., 2008) and that this overlaps with the genetic risk of psychotic disorders (Power et al., 2014). However, as in our study no significant effect of the rs1344706 genotype on cannabis use was detected, a specific gene–environment correlation between *ZNF804A* and cannabis use can be ruled out.

For the interpretation of this GxE interplay, it is essential to understand the biological function of the *ZNF804A* gene and the effect of the polymorphic variant. As mentioned, *ZNF804A* is believed to be involved in the development and function of neural and synaptic structures (Chang et al., 2017), by regulating the expression of other genes, some of which have previously been associated with schizophrenia (Girgenti et al., 2012; Umeda-Yano et al., 2013).

Therefore, it was first considered whether the risk allele affects the expression of *ZNF804A* or even of other genes. Two studies converge and show that the SNP rs1344706 has a cis-acting effect on *ZNF804A* expression in human fetal brain (Hill and Bray, 2012; Tao et al., 2014). Specifically, those studies showed reduced expression associated with the risk allele (A); however, allelic direction still remains controversial due to contradictory results from other studies (Guella et al., 2014; Williams et al., 2011). Nonetheless, it can be hypothesized that dysregulation of *ZNF804A* expression during such a critical period as the prenatal stage, could have an impact on important neurodevelopmental processes and ultimately increase the risk of psychosis by modulating sensitivity to environmental stressors. However, since rs1344706 maps to an intronic region, the mechanisms causing these expression changes remain poorly understood. A recent study proposed the *MYT1L* and *GATA2* genes, which are involved in oligodendrocyte and neuronal differentiation and have previously been associated with schizophrenia, as strong candidates for regulating *ZNF804A* expression via rs1344706 (Hess et al., 2015). However, further efforts are needed to determine the role that rs1344706 plays in regulating *ZNF804A* expression during development.

Some limitations of this study must be acknowledged. First, the participants in this study were university students and therefore generalizing the findings to other populations must be done with caution. Second, the participants were volunteers for the study, which may introduce some selection bias into our sample. Nonetheless, as the students were asked to participate in a study to examine the interaction between different psychological, biological and social factors, they did not know in advance that they would be interviewed specifically on cannabis use and schizotypy, which reduces this possible bias. Third, the cross-sectional design is not the optimum to test causal associations and the retrospective measures may constitute an inherent source of bias. However, the genetic and environmental variables were selected based on previous findings and the analysis had a directional hypothesis, also defined according to the evidence (Cardon and Bell, 2001; Dick et al., 2015). Despite these limitations, the design of this study should be highlighted as an intrinsic strength; the assessment of the association between lifetime cannabis use and schizotypy in a non-clinical sample averts the presence of confounding factors inherent to psychosis, such as medication or the heterogeneous symptomatology of the disorder. Finally, since early age of first cannabis use is associated

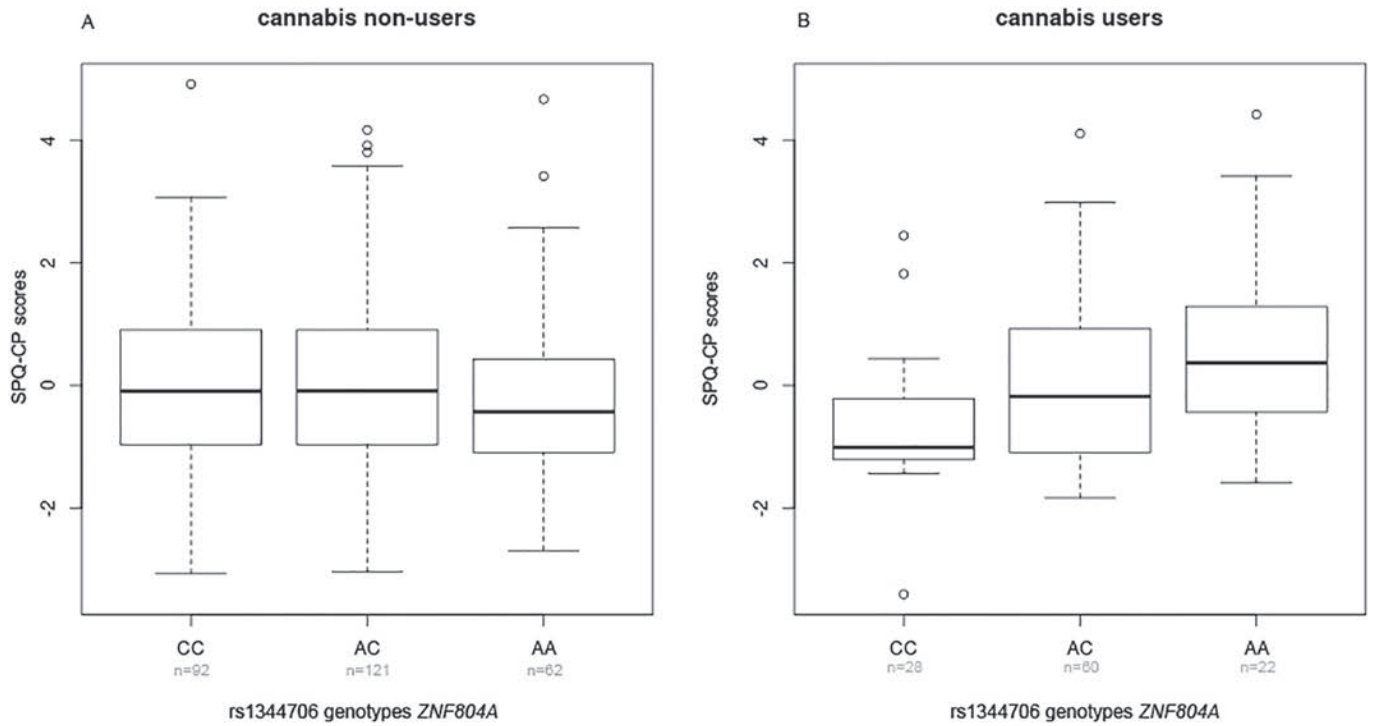

Fig. 1. Effect of *ZNF804A* polymorphism (rs1344706) on SPQ-CP scores in cannabis non-users (A) and users (B). The X axes of the box plots correspond to the three *ZNF804A* genotypes and the Y axes to the residuals obtained from a regression between SPQ-CP scores and the covariates (sex, SCL anxiety scores and the use of other drugs). Linear models with permutation tests showed a significant effect of the polymorphism in cannabis users ($\beta = 0.53$ $p = .009$); while no differences were detected in cannabis non-users ($\beta = 0.14$ $p = .204$).

with an increased risk for schizotypy (Eren et al., 2017) and psychosis (Barrigón et al., 2010), it would have been of value to have the age at onset of cannabis use. Further analyses in independent samples are required to replicate the present results and also to improve our knowledge of the etiological mechanisms underlying the risk of psychosis, both in general population and in clinical samples.

5. Conclusion

In conclusion, our study supports the notion that the gene *ZNF804A* has an effect on psychosis vulnerability (as measured via schizotypy) in young healthy individuals from the general population. In addition, for the first time, we report that the interaction between the *ZNF804A* gene and cannabis use modulates the risk of psychosis. Specifically, we found that this interaction followed a dose–effect relation: cognitive-perceptual schizotypy increases as the frequency of cannabis use does in individuals carrying the AA genotype (rs1344706).

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics statements

Ethical approval was obtained from the local research ethics committees. All the participants provided written consent after being informed of the study procedures and implications. All procedures were carried out in accordance with the Declaration of Helsinki.

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Supervisor's report on the contribution of the PhD applicant to the article

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as the supervisors of the present doctoral thesis by Jordi Soler Garcia, hereby certify that the participation of the PhD applicant in the article *"ZNF804A gene and cannabis use: interaction on the risk for psychosis in a non-clinical sample"* included the following tasks:

- Conception and design of the study
- Statistical analyses
- Writing of the first manuscript draft
- Critical revision of the article for intellectual content

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Barcelona, May 14th 2021

6.

Analysis of *AKT1* and cannabis moderation effects on cognitive performance in healthy subjects

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Abstract

Background: Evidence suggests that the *AKT1* gene may modulate the degree to which cannabis use induces cognitive alterations in patients with a psychotic disorder.

Aim: To examine the interplay between *AKT1* and cannabis use in terms of the cognitive performance of the general population.

Methods: Our sample consisted of 389 Spanish university students. Sustained attention was measured via the Continuous Performance Test–Identical Pairs, immediate and delayed verbal memory with the Logical Memory subtest of the Wechsler Memory Scale, and working memory with the Wisconsin Card Sorting Test. Lifetime cannabis use frequency was assessed and individuals were classified as cannabis users or non-users. Two single nucleotide polymorphisms of the *AKT1* gene were genotyped and, according to previous studies, each subject was defined as a carrier of two, one or no copies of the haplotype (rs2494732(C)–rs1130233(A)). Multiple linear regressions were conducted to test the effect of the genetic variability and cannabis use (and their interaction) on cognitive performance.

Results: An effect of the *AKT1* haplotype was found on attention scores: individuals with two copies of the haplotype performed better (β=0.18, $p<0.001$ (adjusted for false discovery rate)), while neither cannabis nor the *AKT1*–cannabis interaction was associated with attention. No effect of *AKT1*, cannabis or the *AKT1*–cannabis interaction was found on verbal memory or working memory.

Conclusions: Our study provides additional evidence that *AKT1* modulates cognitive performance. However, in our non-clinical sample, the previously reported interaction between cannabis use and the *AKT1* gene was not replicated.

Keywords
Genetics, cannabis, cognition

Introduction

Cognition is a complex phenotypic construct with great heterogeneity across individuals. This phenotype variability within the population is the consequence of many factors, both genetic and environmental, that determine brain formation and neurodevelopment during the foetal stage and first decades of life (Davies et al., 2011; Gräff and Mansuy, 2008; Haworth et al., 2010; Kochhann et al., 2017). Therefore, the genetic makeup of each individual, the influence of different environmental factors and the interaction between them all contribute to the wide range of capacities observed in human cognition.

As a complex phenotypic construct, cognition can be divided into different domains, including attention, memory, learning, executive functions and social cognition. Performance in these domains follows a normal distribution in the general population and shows a certain degree of heritability (20–80%) (Blokland et al., 2017; Greenwood et al., 2007, 2016). Evidence indicates that cognitive disturbances in some domains are characteristic of certain mental disorders, including psychotic disorders. Compared to healthy individuals, people who have been diagnosed with schizophrenia show a broad range of cognitive impairments and statistically demonstrate a decrease of some 1–2 standard deviations in tests of several cognitive domains, including working, verbal and visual memories, processing speed, attention, social cognition and intelligence (Green et al., 2004; Keefe and Harvey, 2012; Swerdlow et al., 2015). The fact that these impairments exist before the onset of illness and that they are also present to a lesser extent in healthy twins and other relatives of patients (Keefe and Harvey, 2012; Núñez et al., 2016) has led to them being considered valuable endophenotypes that are indicative of an increased risk of schizophrenia. Therefore, it is suggested that focusing on

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cognitive endophenotypes might be a useful approach in the identification of genetic factors associated not only with disease mechanisms but also with the underlying biological processes of cognitive functions (Mark and Toulopoulou, 2016; Swerdlow et al., 2015). For instance, a recent genome-wide analysis based on cognitive endophenotypes for schizophrenia has reported the involvement of genes related to neurodevelopmental processes in attentional deficits (Greenwood et al., 2019).

The *AKT1* gene (14q32.32) encodes a serine/threonine kinase (Akt serine/threonine protein kinase 1, Akt1) involved in the PI3K/GSK-3 pathway (Scheid and Woodgett, 2001, 2003) which regulates multiple cellular processes, including transcription, apoptosis, stress response, cell proliferation and cell survival (Scheid and Woodgett, 2001). This kinase has also been implicated in a variety of neurodevelopmental functions such as neuronal migration, growth factor dependent survival of neurons, axon growth and branching, as well as placental function processes (Howell and Law, 2019).

More specifically, Akt1 is involved in modulating synaptic dopaminergic transmission systems, where it is a key signalling intermediate downstream of dopamine receptor D2 (DRD2). The relationship between Akt1 and D2 receptor signalling has been elucidated by data indicating that DRD2 stimulation by dopamine inhibits Akt1 signalling through the β-arrestin 2/phosphatase PP2A complex dephosphorylation (Beaulieu et al., 2007a, 2007b). Furthermore, clozapine, a D2 antagonist antipsychotic medication, exerts its effects, at least in part, through modulation of levels and activity of Akt1 and GSK-3β (Freyberg et al., 2010).

According to this role of Akt1 in dopaminergic signalling, and taking into account that optimal execution of cognitive tasks critically depends on proper levels of dopamine within the prefrontal cortex (O'Reilly, 2006; Seamans and Yang, 2004; Tan et al., 2007), it has been suggested that differential *AKT1* gene expression could modulate cognition through the regulation of dopaminergic neurotransmission. In this sense, some *AKT1* gene polymorphic variants (rs1130233 and rs1130214) have been linked to gene expression changes in human lymphoblasts (Tan et al., 2008) and to Akt1 protein level reductions in peripheral lymphocytes and in postmortem prefrontal cortex of patients with schizophrenia (Emamian et al., 2004; Thiselton et al., 2008). Moreover, in healthy subjects the interaction between *AKT1* (rs1130233) and *DRD2* (rs1076560) has been related to reduced Akt1 protein levels in peripheral blood mononuclear cells, as well as with altered cingulate cortex activity during attentional control (functional magnetic resonance imaging) and reduced accuracy in the performance of a sustained attention task (Continuous Performance Test, CPT) (Blasi et al., 2011).

In addition, the *AKT1* and *DRD2* genes have been both associated with cognitive performance in different domains, including attention, verbal learning and verbal memory (Klaus and Pennington, 2019; Ohi et al., 2013; Pietiläinen et al., 2009; Tan et al., 2008; Van Winkel et al., 2011b). As regards the risk for schizophrenia, candidate gene approaches have highlighted the involvement of both genes (Bajestan et al., 2006; Blasi et al., 2011; Edwards et al., 2016; Ikeda et al., 2004; Karege et al., 2010, 2012; Norton et al., 2007; Schwab et al., 2005; Thiselton et al., 2008; Xu et al., 2007), while genome-wide data have indicated the association of *DRD2* gene (Ripke et al., 2014).

With respect to the role of environmental factors on cognitive performance, the relationship between cannabis and cognition has been extensively studied in healthy individuals, as well as in those with a diagnosis of schizophrenia. Despite several studies associate cannabis use with poorer cognitive performance (Castellanos-Ryan et al., 2017; González-Pinto et al., 2016; Nader and Sanchez, 2018; Núñez et al., 2016; Thames et al., 2014), others show opposite or mixed results (Becker et al., 2018; Meijer et al., 2012; Rabin et al., 2011; Sánchez-Torres et al., 2013; Schoeler et al., 2016; Segev and Lev-Ran, 2012; Yucel et al., 2012). Cannabis use has also been associated with the emergence of psychotic symptoms (Minozzi et al., 2010; Moore et al., 2007), and a meta-analysis suggested a dose–response relationship between the intensity of cannabis use and the risk of psychosis (Marconi et al., 2016).

However, it is of note that there is a marked interindividual variability in the susceptibility to cannabis effects, which is thought to have a genetic basis. In this view, individuals who are genetically vulnerable to psychosis show increased cannabis-induced psychotic symptoms (D'Souza et al., 2005; Henquet et al., 2005; Van Os et al., 2002; Verdoux et al., 2003), while a family history of psychosis has also been reported to influence the cannabis use–derived effects on cognitive performance (González-Pinto et al., 2016; Henquet et al., 2006). Based on the demonstrated effect of tetrahydrocannabinol (THC) on central dopaminergic transmission in animal studies (Murray et al., 2007), it is thought that genes influencing the transmission or metabolism of brain dopamine can be involved in such interindividual differences. In this regards, experimental data have shown the moderation of the psychotomimetic effects of THC and their neurophysiological underpinnings by *AKT1* gene variability (Bhattacharyya et al., 2012) and have also reported that the Akt1 pathway can be activated by THC (Ozaita et al., 2007; Sánchez et al., 2003). Also, different studies have shown that the interaction between the *AKT1* gene and cannabis use modulates cognitive alterations (Bhattacharyya et al., 2014; Morgan et al., 2016; Van Winkel et al., 2011b) and influences the risk of psychosis (Di Forti et al., 2012; Van Winkel et al., 2011a), in both healthy subjects and individuals with a diagnosis of schizophrenia.

According to all the above, we hypothesized that polymorphic variants of the *AKT1* gene might explain part of the observed variance in cognitive performance of healthy subjects and that this association might be modulated by cannabis use. Therefore, to extend the knowledge of the relationship between cannabis use and cognitive performance variability in a sample of healthy individuals, we here have studied: (a) the association of the *AKT1* gene with different cognitive dimensions; and (b) whether lifetime cannabis use modulates that association.

Methods

Sample

Our sample consisted of 389 subjects from the Spanish general population who were recruited in 2004–2005 at the campus of the Jaume I University in Castelló (Spain). Exclusion criteria were any major medical illness affecting brain function, neurological disorders, a personal history of head injury or psychiatric medical

Table 1. Information of *AKT1* single nucleotide polymorphisms (SNPs) included in this study.

AKT1 gene SNPs	Chr.	Chr. position	Gene position	Alleles[a]	MAF_{1000G}[b]	MAF_{sample}[c]	Genotype frequency (%)		
rs2494732	14	104772855	Intron 10	T/C	0.44	0.411	TT (36.1%)	TC (45.6%)	CC (18.3%)
rs1130233	14	104773557	Exon 8 (synonymous variant)	G/A	0.243	0.255	GG (55.7%)	GA (37.7%)	AA (6.6%)

The table includes the dbSNP number, the genomic and gene position and the alleles of each SNP (UCSC Genome Browser on Human March 2006 Assembly (GRCh.38), http://genome.ucsc.edu/cgi-bin/hgTracks). Observed genotypic and allelic frequencies are also given.

[a]The less frequent allele (minor allele) is placed second.

[b]MAF_{1000G} refers to the minor allele frequency from the 1000 Genomes Project Phase 3 across European populations (Abecasis et al., 2012).

[c]MAF_{sample} refers to the minor allele frequency observed in the current sample.

treatment and a non-European ancestry. These were screened by trained psychologists using a short interview designed for this study that included selected items from psychiatric diagnosis structured scales, such as the Structured Clinical Interview for DSM-IV (SCID-I) (First et al., 1999) and the Familiar Interview for Genetic Studies (FIGS) (Maxwell, 1992). The participants were asked specific questions regarding psychiatric treatment, psychotropic medication, hospital admissions and suicide attempts.

Ethical approval was obtained from local research ethics committees. All the participants provided written consent after being informed of the study procedures and implications. All procedures were carried out in accordance with the Declaration of Helsinki.

Measures

Attention and both verbal and working memory were selected as the cognitive outcome measures based on previous studies of patients with psychosis and healthy controls that reported the effects of cannabis use (Henquet et al., 2006; Van Winkel et al., 2011b). Sustained attention was measured using the Continuous Performance Test–Identical Pairs (CPT-IP) (Cornblatt et al., 1988). The composite score derived from the CPT-IP is the signal detection index (d′, calculated as the ratio between correct hits/responses and false alarms), which reflects the ability of the participant to discriminate between signal and noise. We calculated d′ separately for the shapes and digits conditions of the test. Higher values of d′ indicate better performance. Immediate and delayed verbal memory was assessed using the Logical Memory subtest of the Wechsler Memory Scale (WMS-R) (Wechsler, 1997b) , and we used percentile scores. Working memory was assessed via the Wisconsin Card Sorting Test (Heaton, 1981) and the number of perseverative errors was selected as the outcome measure. Higher numbers of errors indicate lower performance. Finally, intelligence quotient (IQ) was estimated using the Block Design and Information subtests of the WAIS-III (Wechsler, 1997a), following the method suggested by Sattler (2001).

Lifetime cannabis use was assessed via a question regarding the frequency of consumption: the participants were asked about whether they used cannabis ‘never’, ‘occasionally’ (at some time during their life), ‘monthly’, ‘weekly’ or ‘daily’. This variable was then dichotomized as cannabis use (daily, weekly and monthly) or no cannabis use (occasionally and never), differentiating those with an abuse/dependence pattern from those with a no-use or non-regular-use pattern.

Similarly, the participants were asked about their use of other illicit drugs (amphetamine-type stimulants, cocaine, heroin and other opioids, and ecstasy) and were classified as users of other drugs (daily, weekly and monthly) or non-users of other drugs (sporadically or never). Cannabis use information was available for all the participants and data on other drugs were available for 99% of the sample.

The Community Assessment of Psychic Experiences (CAPE, Stefanis et al., 2002) is a self-report questionnaire that measures the lifetime prevalence of psychotic experiences on a frequency scale ranging from ‘never’ to ‘nearly always’. The CAPE provides a continuous score (higher scores being indicative of more prevalent experiences) in two dimensions. The positive dimension mainly includes items referring to subclinical expressions of positive psychotic symptoms (hallucinations and delusions), such as: ‘Do you ever feel as if things in magazines or TV were written especially for you?’ The negative dimension includes items assessing subclinical expressions of negative psychotic symptoms (such as alogia, avolition, anhedonia and a lack of interest in social relationships), such as: ‘Do you ever feel that you experience few or no emotions at important events?’ Dimensions of psychic experiences assessed with the CAPE have been shown to be stable, reliable and valid (Konings et al., 2006); furthermore, the scale has been validated in the general Spanish population (Ros-Morente et al., 2011).

Molecular analysis

Genomic DNA was extracted from each individual via buccal mucosa samples by means of a cotton swab using the BuccalAmp DNA Extraction Kit (Epicentre® Biotechnologies; Madison, WI, USA).

In accordance with the previous gene–environment interaction studies mentioned above (Di Forti et al., 2012; Van Winkel et al., 2011b), two single nucleotide polymorphisms (SNPs) of the *AKT1* gene were determined using Applied Biosystems TaqMan 5’ exonuclease assays: rs2494732 and rs1130233 (Table 1). Polymerase chain reaction plates were read on an ABI PRISM 7900HT instrument with SDS v2.1 software (Applied Biosystems; Foster City, CA, USA). The genotyping call rate for both SNPs was >99%. The accuracy of the method was tested by re-genotyping 10% of the samples and confirming all the repeated genotypes.

Bearing in mind the previous studies based on *AKT1* haplotypes as well as the higher accuracy and statistical power of the haplotype analyses compared with approaches based on one SNP, we developed an association analysis of the haplotype of

the two *AKT1* SNPs. The program Haploview v4.1 (Barrett et al., 2005) was used to estimate the linkage disequilibrium (LD) between the two SNPs. Taking into account the LD pattern detected in our sample (D′=0.95, r^2=0.50), individual haplotypes were estimated using UNPHASED v3.0.13 (Dudbridge, 2003). Considering only those haplotypes estimated with a probability ⩾95% (n=382), each subject was defined as a carrier of two, one or no copies of the haplotype (rs2494732(C)–rs1130233(A)). This haplotype was selected according to the results of previous studies which show that the C allele of the SNP rs2494732 and the A allele of the SNP rs1130233 are associated with neurocognitive performance (Tan et al., 2008; Van Winkel et al., 2011a).

Statistical analyses

All data were processed using PASW statistics (SPSS Inc.; Chicago, IL, USA) and Stata v.14 (StataCorp 2015, College Station, TX, USA: StataCorp LP).

The normality of the distribution was tested for IQ, CAPE and mean cognitive scores and all of them showed a normal distribution ($p>0.05$). Student's t-test and analysis of variance were used to compare the means of continuous variables between two or more groups, respectively. A chi-squared test was performed to analyse the distribution of qualitative variables between groups and to examine the Hardy–Weinberg equilibrium.

We conducted multiple linear regressions to test the effect of genetic and environmental factors, and their interaction, on cognitive scores. As an initial model, the main effect of the independent variables (haplotype and cannabis use) on cognitive scores was tested. The effect of the interaction between haplotype and cannabis use on cognitive factors was added to the same model as a second step. Analyses were performed twice, first, using the dichotomized cannabis use variable (cannabis users and non-users) and, second, using the frequency of cannabis use ('never', 'occasionally', 'monthly', 'weekly' or 'daily') in order to test whether there is a dose–response effect of cannabis use. Sex, age, IQ, CAPE positive and negative scores, and the use of other drugs were included as covariates. We corrected the results for multiple comparisons using the false discovery rate (FDR) correction within each cognitive test (Benjamini and Hochberg, 1995). FDR-adjusted p-values <0.05 were considered statistically significant.

Results

Sample description

Participants were university students (43% males) with a mean age at interview of 21.46 years (SD=3.09). The mean IQ was 99.18 (SD=13.69). The CAPE positive and negative scores were 1.32 (SD=0.21) and 1.63 (SD=0.35), respectively. The mean cognitive scores are given in Table 2. Since the measure of the perseverative error had a positively skewed distribution, a 1/square root transformation was first applied.

A total of 71% of participants were classified as non-cannabis users (35% never and 36% occasionally); with 29% classified as cannabis users (11% monthly, 11% weekly and 7% daily). Use of other drugs was reported by 4.5% of individuals.

The genotype distribution is shown in Table 2. None of the genotype frequencies deviated significantly from Hardy–Weinberg equilibrium ($p>0.05$). The observed minor allele frequencies of both SNPs were similar to those reported by the 1000 Genomes Project for populations with European ancestry (Abecasis et al., 2012).

In our analysis of the effect of the covariates (sex, age, IQ and CAPE scores) on mean cognitive scores, significant differences between women and men were observed for d′shapes (t=−5.30 $p<0.001$) and d′numbers (t=−5.40, $p<0.001$). IQ correlated significantly with all the neurocognitive measures ($p<0.001$).

When cannabis non-users were compared with cannabis users, no statistically significant differences were observed for age, genotype, IQ or CAPE scores. Lifetime cannabis consumption was significantly more prevalent in men, 34.7%, than in women, 24.8% ($\chi 2$ =4.80, df=1, p=0.02).

Cognitive performance

Concerning sustained attention, a main effect of the *AKT1* haplotype (rs2494732–rs1130233) was observed for d′shapes (β=0.18, standard error=0.059, FDR-adjusted $p<0.001$): individuals with two copies of the haplotype C-A presented better scores (Table 3). Neither cannabis use nor the *AKT1*–cannabis interaction was associated with attention measures (FDR-adjusted $p>0.05$).

With respect to verbal and working memory, no major effect of *AKT1*, cannabis or *AKT1*–cannabis interaction was found on these measures (FDR-adjusted $p>0.05$).

Discussion

This study provides additional evidence of the effect of the *AKT1* gene on cognitive performance in healthy subjects. Specifically, we observed an association between the haplotype rs2494732–rs1130233 and sustained attention: individuals with two copies of the haplotype (C-A) performed better in the d′shapes measure of the CPT-IP.

With regards to the effect *AKT1* gene has on attention measures, the three studies that previously assessed this matter show mixed results (Ohi et al., 2013; Tan et al., 2008; Van Winkel et al., 2011b). On the one hand, Tan et al. (2008) and Van Winkel et al. (2011b) did not find any association between *AKT1* and sustained attention (CPT measures) in a sample of healthy individuals or a sample of patients with psychotic disorders, siblings and unrelated controls, respectively. However, Ohi et al. (2013) reported that patients with schizophrenia homozygous for the allele C (rs2494732) demonstrated better attentional performance (d′numbers, CPT-IP) than A allele carriers, while no differences were observed within controls. Interestingly, this same study reported a brain morphological association of this polymorphism with reductions of grey matter volumes in the right inferior parietal lobule, which is related to attentional processes. Despite the different natures of the samples, those results are in line with the findings of our present study, in which the C allele of rs2494732 is included in the haplotype associated with higher d′shapes scores. Similarly, concerning rs1130233, Tan et al. (2008) found that this polymorphism was involved with inefficient processing in the prefrontal cortex in healthy individuals. The same polymorphism was also associated with connectivity deficits between

Table 2. Sample description.

		Attention		Immediate verbal memory	Delayed verbal memory	Working memory
		CPT (d'shapes)	CPT (d'numbers)	Logical Memory, Wechsler Memory Scale (percentile scores)	Logical Memory, Wechsler Memory Scale (percentile scores)	Wisconsin Card Sorting Test (perseverative errors)
	n=382	1.80 (0.71) [0–4.25]	1.71 (0.80) [0.16–3.96]	51.85 (26.52) [1–99]	51.49 (24.74) [1–99]	7.79 (5.71) [3–40]
Sex	Female (*n*=217)	1.64 (0.64) [0–3.41][a]	1.53 (0.68) [0.16–3.96][a]	53.26 (25.45) [2–99]	53.05 (23.05) [1–98]	7.98 (6.16) [3–38]
	Male (*n*=165)	2.00 (0.76) [0–4.25][a]	1.96 (0.88) [0.19–3.96][a]	50.00 (27.83) [1–99]	49.44 (26.74) [1–99]	7.54 (5.09) [3–40]
AKT1 haplotype rs2494732(C)–rs1130233(A)	0 copies (*n*=217)	1.70 (0.64) [0–3.34][b]	1.64 (0.77) [0.16–3.96]	51.86 (25.64) [4–99]	51.43 (25.00) [4–99]	8.05 (5.91) [3–39]
	1 copy (*n*=139)	1.87 (0.76) [0–3.62][b]	1.80 (0.84) [0.17–3.96]	52.38 (26.95) [1–98]	51.87 (23.99) [1–97]	7.59 (5.60) [4–40]
	2 copies (*n*=26)	2.23 (0.81) [0.5–4.25][b]	1.87 (0.82) [0.44 – 3.66]	48.96 (31.94) [3–96]	49.88 (27.36) [7–95]	6.73 (4.53) [3–23]
Cannabis use (frequency of use)	Never use (*n*=135)	1.76 (0.70) [0–3.34]	1.65 (0.79) [0.26–3.67]	52.24 (26.75) [2–99]	51.04 (23.90) [4–99]	8.02 (6.08) [4–40]
	Occasionally (*n*=136)	1.85 (0.66) [0.23–4.25]	1.82 (0.79) [0.16–3.96]	54.50 (26.68) [3–97]	55.16 (24.90) [5–98]	7.02 (4.19) [3–34]
	Monthly (*n*=41)	1.86 (0.73) [0–3.41]	1.72 (0.74) [0.39–3.96]	46.14 (26.45) [7–94]	44.51 (24.47) [12–88]	7.31 (4.99) [3–32]
	Weekly (*n*=45)	1.83 (0.78) [0.33–3.41]	1.72 (0.94) [0.19–3.96]	48.44 (26.03) [3–97]	48.11 (25.36) [1–95]	8.77 (6.34) [4–32]
	Daily (*n*=25)	1.59 (0.89) [0.29–3.41]	1.51 (0.68) [0.17–3.23]	50.84 (25.28) [1–90]	51.44 (26.11) [1–94]	9.68 (9.34) [3–38]
Cannabis use (dichotomous)	No use	1.80 (0.68) [0.00–4.20]	1.73 (0.79) [0.16–3.96]	53.3 (26.6) [2–99]	53.1 (24.4) [4–99]	7.52 (5.24) [3–40]
	Use	1.79 (0.79) [0.09–3.41]	1.67 (0.81) [0.17–3.96]	48.1 (25.8) [1–97]	47.5 (25.1) [1–95]	8.44 (6.71) [3–38]

Performance on cognitive tests is given according to sex, haplotypes and cannabis use. Mean value, standard deviation (in parentheses) and range (in square brackets) are reported in each case.
[a]Significant differences between females and males on d'shapes and d'numbers ($t(380)=5.32$ $p<0.001$ and $t(380)=4.96$, $p<0.001$, respectively).
[b]Significant differences between main effect of the haplotype on d'shapes ($F(6, 371)=7.47$, $p<0.001$).
CPT: Continuous Performance Test; d': signal detection index.

Table 3. Results of the multiple linear regression conducted to test the interaction between the *AKT1* haplotype (rs2494732(C)–rs1130233(A); 0, 1 or 2 copies) with cannabis use (dichotomous) on d'shapes score (from Continuous Performance Test, CPT-IP).

	Beta	SE	*p*-value	FDR-adjusted *p*-value
Main effects				
Haplotype	0.179	0.059	<**0.001**	<**0.001**
Cannabis	–0.036	0.081	0.477	0.741
Covariates				
Age	–0.067	0.011	0.165	0.330
Sex	–0.159	0.075	**0.002**	**0.005**
IQ	0.272	0.002	<**0.001**	<**0.001**
CAPE positive score	0.320	0.179	0.556	0.741
CAPE negative score	–0.007	0.109	0.885	0.885
Other drugs use	0.203	0.081	0.695	0.794
Interaction				
0 copies of the haplotype - cannabis use	–0.019	0.312	0.903	0.903
1 copy of the haplotype - cannabis use	–0.028	0.168	0.678	0.678
2 copies of the haplotype - cannabis use	–0.005	0.303	0.929	0.929

CAPE: Community Assessment of Psychic Experiences; FDR: false discovery rate; IQ: intelligence quotient; SE: standard error.

the prefrontal and subcortical regions during working memory manipulation also in healthy subjects (Tan et al., 2012). Then, our study extends the evidence for a putative role of *AKT1* within the mechanisms underlying sustained attention. However, further studies are needed in order to verify whether this relation also occurs in other general population samples.

Concerning the cannabis main effect on cognition, despite basing our hypothesis on evidence showing the relationship between cannabis use and cognitive deficits in healthy individuals (Broyd et al., 2016; Castellanos-Ryan et al., 2017; Nader and Sanchez, 2018; Thames et al., 2014), in our sample we did not detect such an effect on any of the evaluated cognitive dimensions. However, other studies performed in healthy young adult samples have also reported no significant associations between cannabis use and cognitive measurements (Cousijn et al., 2014; Sánchez-Torres et al., 2013; Scholes and Martin-Iverson, 2010; Van Winkel et al., 2011b). Such discrepancies should probably be interpreted in terms of the difficulty in controlling for the different factors that have been indicated as influencing the effects of cannabis on neurocognitive performance (Cosker et al., 2018). Along these lines, despite having controlled for some variables such as age, sex and concomitant use of other psychoactive drugs, the role of the specific dose, the type of cannabis or the extent of prior cannabis exposure could not be tested in our sample.

Finally, in relation to the *AKT1*–cannabis interaction on cognition, our data based on a non-clinical sample are in agreement with the results of Van Winkel et al. (2011b), who did not find a significant interplay between *AKT1* and cannabis use on attention measures in healthy individuals contrary to the significant interaction they observed in patients with schizophrenia. More specifically, they reported that individuals with schizophrenia who carried the CC genotype at the SNP rs2494732 performed worse than individuals with the TT genotype in attention tests after cannabis use, while within healthy subjects such an effect was not detected. Other studies have also shown that cannabis use might interact with genetic loading in such a way as to moderate cognitive performance in schizophrenia patients (González-Pinto et al., 2016; Henquet et al., 2006). Nevertheless, data on healthy subjects are scarce and further interaction studies should be performed to understand better the effect of the relationship between *AKT1* variability and cannabis use on cognition. Such studies should take into account the trade-off between sample size and measurement precision (Bhattacharyya et al., 2014; Di Forti et al., 2012; Van Winkel et al., 2011a, 2011b), and they should also include markers of clinical and genetic vulnerability.

Some limitations of our study must be acknowledged. First, the participants were all university students and generalization to other populations must be proceeded with caution. Second, the participants were self-referred for the study, which may have introduced some selection bias into our sample. Nonetheless, as the students were asked to participate in a study to examine the interaction between different psychological, biological and social factors, they did not know in advance that they would be interviewed specifically concerning cannabis use, which reduces this possible bias. Third, despite our measure of cannabis use being considered to have a fair level of confidence, the retrospective assessment constitutes an inherent source of bias. Concerning cannabis use evaluation, as mentioned above, the lack of availability in our sample of the age of initiation, the type of cannabis used, the amount consumed, and the last time of consumption reduces the precision of our measure. As a counterpart, the analyses tested a possible dose–effect relationship of cannabis use on the evaluated measures. Finally, a cross-sectional design is not optimal for testing causal associations. However, the genetic and environmental variables were selected based on previous findings and the analyses involved a directional hypothesis that was defined following the previous evidence (Bhattacharyya et al., 2014; Di Forti et al., 2012; Van Winkel et al., 2011a, 2011b).

Despite these limitations, the design of this study provides it with intrinsic strength. Assessment of the association between cannabis use and neurocognitive function in a non-clinical sample overcomes the problems associated with the presence of multiple confounding factors that are inherent to psychosis, such as medication or the heterogeneous symptomatology of the disorder. In this sense, we include the CAPE dimension scores in the statistical models to exclude the confounding factors mediated by psychosis vulnerability. Further analyses of independent samples are required to replicate the present results and to improve knowledge

of the aetiological mechanisms underlying cognitive performance, both in the general population and in clinical samples.

In conclusion, our study supports the notion that the *AKT1* gene influences sustained attention in healthy young individuals from the general population, while it does not replicate the previously reported interaction of the *AKT1* gene with cannabis use.

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Supervisor's report on the contribution of the PhD applicant to the article

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as the supervisors of the present doctoral thesis by Jordi Soler Garcia, hereby certify that the participation of the PhD applicant in the article *"The effect of the AKT1 gene and cannabis use on cognitive performance in healthy subjects"* included the following tasks:

- Conception and design of the study
- Statistical analyses
- Writing of the first manuscript draft
- Critical revision of the article for intellectual content

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Barcelona, April 23rd 2021

GLOBAL SUMMARY OF RESULTS

The three specific hypotheses were tested throughout three different aims:

Aim I. To assess the **familiality** of clinical (schizotypy), cognitive (executive function, reasoning skills, attention, memory, working memory) and neurodevelopmental (dermatoglyphics) intermediate phenotypes of interest in families with at least one patient with an offspring diagnosed with a psychotic disorder, and to develop a methodology to estimate continuous score (**intrafamilial resemblance score, IRS**) that estimates the similarity of these traits among family members.

The articles derived from these aims are:

- **Soler J et al., 2017**. Familial Aggregation of Schizotypy in Schizophrenia-Spectrum Disorders and Its Relation to Clinical and Neurodevelopmental Traits. *Journal of Psychiatric Research*, 2017 Jan; 84:214-220.

- **Soler J et al., 2020**. Familial aggregation analysis of cognitive performance in early-onset bipolar disorder. *European Society of Child and Adolescent Psychiatry*, 2020 Dec; 29(12):1705-1716.

Referring to this aim, the following **results** were obtained:

I. Schizotypy is a familial phenotype (ICC=0.30 $p<0.001$) and the estimation of the intrafamilial resemblance score (IRS) allowed the identification of families with high or low resemblance for this phenotype. Moreover, we observed that healthy relatives with higher schizotypy values presented a higher prevalence of ridge dissociations, another psychosis liability marker. We also described that the schizotypy aggregation pattern in relatives might be predictive of patients' clinical characteristics as patients from families with higher schizotypy scores showed more severe disorganised symptoms at the psychotic episode ($p=0.035$) and 1 year later ($p=0.011$) than other patients. Besides adding evidence to the role of schizotypy as a putative vulnerability marker for psychotic disorders that runs within families, these

results showed for the first time the value of estimating the IRS to disentangle the clinical heterogeneity between families.

II. Attention and working memory dimensions are potential familial liability markers for early-onset bipolar disorder. Then, they could be used to distinguish different familial subgroups in terms of genetic aetiology. In this regard, the attention and working memory (AW) dimension showed to be familial (ICC=0.37 p=0.0004), and the estimation of the intrafamilial resemblance score (IRS) allowed identifying families whose members share low AW scores and families with all members showing high scores. Thus, these results again suggest that the estimation of the IRS might represent a valuable strategy to facilitate the interpretation of the clinical heterogeneity of psychotic disorders and define aetiological subgroups of families with these disorders (in this case, EOBD).

In summary, the findings reported in these manuscripts support our hypothesis, suggesting that the estimation of the intrafamilial resemblance score (IRS) and its application to the analysis of the familial aggregation of intermediate phenotypes (including schizotypy, dermatoglyphic and cognitive functions) is a valuable strategy to facilitate the identification of more homogeneous individuals with higher liability risk to psychotic disorders.

Aim II. To study the correlates of **genetic variants** in genes encoding for different synapse function and regulation proteins (***DAOA*, *RGS4*, *AKT1, ZNF804A*)** with clinical (**schizotypy**), neurodevelopmental (**dermatoglyphics**) and **cognitive** intermediate phenotypes of interest in psychotic disorders.

In relation to this, we also aimed to conduct a systematic review of the literature to analyse the involvement of particular genetic variants within genes encoding for scaffolding proteins across psychiatric diagnoses.

The articles derived from these aims are:

- **Soler J et al., 2016.** Influence of *DAOA* and *RGS4* genes on the risk for psychotic disorders and their associated executive dysfunctions: A family-based study. *European Psychiatry*, 2016 Feb; 32:42-7.

- **Soler J et al., 2018.** Genetic variability in Scaffolding Proteins and the risk for schizophrenia and autism spectrum disorders: a systematic review. *Journal of Psychiatry & Neuroscience*, 2018 May 28; 43:223–244

- **Soler J et al., 2019**. *ZNF804A* gene and cannabis use: interaction on the risk for psychosis in a non-clinical sample. *Progress in Neuropsychopharmacology & Biological Psychiatry*, 2019 Mar 8; 89:174-180.

- **Fatjó-Vilas M and Soler J, et al., 2019.** Analysis of *AKT1* and cannabis moderation effects on cognitive performance in healthy subjects. Journal of Psychopharmacology, 2020 Sep; 34(9):990-998

Referring to this aim, the following **results** were obtained:

I. *DAOA* gene might contribute to the risk for psychotic disorders through the modulation of executive function, probably through deregulating the glutamatergic signalling. More concretely, the analyses showed that the haplotype GAGACT at *DAOA* was under-transmitted to patients (p=0.0008), indicating its association with these disorders. With regards to cognitive performance, the *DAOA* haplotype GAGGCT was associated with worse scores in TMT-B (p=0.018) in patients with schizophrenia only. *RGS4* analyses did not report significant results.

II. Genetic variants in scaffolding genes are pleiotropic and contribute to the shared genetic liability across these disorders, probably throughout the deregulation of the synaptic functioning. In this sense, we found that patients with schizophrenia or autism-spectrum disorders share several genetic variants within these genes, mainly CNV, and that most of these variations also exert an effect on cognitive functions. Moreover, data from

gene expression and animal model-based studies also supported the importance of scaffolding proteins in the correct functioning of the brain and, alternatively, in the appearance of psychotic phenotypes.

III. Genetic variability at the *ZNF804A* gene is associated with the interpersonal dimension of schizotypy (p=0.001), measured with Schizotypal Personality Questionnaire-Brief (SPQ-B). No effect of genotype was observed on the cognitive-perceptual or disorganized dimensions of schizotypy.

IV. The *AKT1* gene modulates neurocognitive performance probably through the regulation of the dopaminergic and glutamatergic neurotransmission systems. Specifically, a main effect of *AKT1* was found on attention scores, measures with the two measures of the continuous performance task (d' shapes and d' numbers). Regarding d' shapes, a main effect of *AKT1* was found (rs2494732: p=0.003; rs1130233: p<0.001; risk haplotype: p<0.001). As regards to the d' digits, the rs2494732 did not show any effect, while the rs1130233 and the risk haplotype did so (rs1130233: β=0.10 SE=0.062 p=0.029; risk haplotype: β=0.10 SE=0.062 p=0.026).

In summary, the findings reported in these manuscripts agree with hypothesis B, as we have evidenced the association between genetic variability in synaptic-related genes with psychosis and schizophrenia-associated intermediate-phenotypes, including cognitive function and schizotypy, in patients with psychotic disorders and healthy individuals from the general population.

Aim III. To study the effect of **cannabis use** on the modulation of the genotype-phenotype correlations in **non-clinical samples**. Particularly, this aim included assessing whether cannabis use mediates the relationship between the *ZNF804A* gene and schizotypy and the relationship between the *AKT1* gene and cognitive function.

The articles derived from this aim are:

- **Soler J et al., 2019**. *ZNF804A* gene and cannabis use: interaction on the risk for psychosis in a non-clinical sample. *Progress in Neuropsychopharmacology & Biological Psychiatry*, 2019 Mar 8; 89:174-180.

- **Fatjó-Vilas M, Soler J, et al., 2019.** Analysis of *AKT1* and cannabis moderation effects on cognitive performance in healthy subjects. Journal of Psychopharmacology, 2020 Sep; 34(9):990-998

Referring to this aim, the following **results** were obtained:

I. The *ZNF804A* gene is a modifying factor of the relationship between a well-known psychosis environmental risk factor such as cannabis and the levels of schizotypy in a non-clinical sample. We particularly detected the interplay between the polymorphism rs1344706 at *ZNF804A* and cannabis use on the cognitive-perceptual dimension of schizotypy ($p=0.005$). Moreover, we also noticed a significant main effect of cannabis use on the disorganised dimension of schizotypy ($p=0.029$).

II. When we investigated the interplay between *AKT1* and cannabis use in cognitive performance (attention and working memory) in the general population, neither cannabis nor *AKT1*-cannabis interaction was associated with any cognitive dimension ($p>0.05$).

In summary, we found that a *ZNF804A*-cannabis interaction might be involved in the variability of a clinical marker such as schizotypy. In contrast, such interplay was not observed between *AKT1* gene and cannabis on cognitive performance. These results illustrate that the study of the relationship between genes variability and cannabis use on psychosis-associated intermediate phenotypes on non-clinical subjects might help to understand the role of these factors in disease.

DISCUSSION

Psychotic disorders constitute multidimensional psychiatric conditions with a tremendous personal, economic and social burden. Despite continuing progress, the aetiological and pathophysiological underpinnings related to psychosis remain largely undetermined and there is no measurable biological marker leading to a robust diagnosis or treatment selection. Indeed, current treatments continue to have significant side effects and inconsistent efficacy across patients. Accordingly, a better understanding of the aetiology of psychosis must be acquired in order to improve the medical's care of patients with these disorders.

With the hope that this research will one day have a clinical transfer intended to improve the life of people with mental disorders, this thesis aimed to provide knowledge about the aetiology of psychosis using different strategies that contribute to unravel the clinical heterogeneity and the complex gene-environment interactions underlying them. Specifically, three hypotheses have been tested, resulting in six published articles. Conclusions derived from these studies are discussed below.

As introduced in section 1.5, there is compelling evidence that the genetic architecture of schizophrenia and other psychotic disorders is pleiotropic and polygenic. In the last decade, there has been remarkable progress in the genetics of psychotic disorders, and the increased collaboration between researchers to achieve large cohorts in which to detect genetic variants associated with these pathologies has been successful (Ripke *et al.*, 2014; Pardiñas *et al.*, 2018; Stahl *et al.*, 2018; Mullins *et al.*, 2020; Ripke, Walters and O'Donovan, 2020). However, one of the main difficulties that GWAS studies have to deal with is the high clinical heterogeneity (both between- and within-patients), which is inherent to schizophrenia and other psychotic disorders. The clinical heterogeneity observed in subjects with the same diagnosis is so significant to the point that the validity and reliability of the categorical diagnostic approaches have been questioned (Allardyce *et al.*, 2007; Craddock and Owen, 2007, 2010; Peralta and Cuesta, 2007, 2008; Esterberg and Compton, 2009; Owen, 2014; Russo *et al.*, 2014; Peralta *et al.*, 2015). In this respect, although GWAS studies conducted on large samples have significantly contributed to identifying the genetic load of psychotic disorders, they do not take this clinical heterogeneity into account.

Accordingly, in order to further advance in the identification of the genetic variants underlying psychotic disorders, we need first to reduce the clinical heterogeneity beneath these disorders. In this sense, a decisive step towards identifying accurate genotype-phenotype correlations is the use of more homogeneous samples regarding their genotype/phenotype. In the present thesis, we hypothesized that the combined use of family-based designs and intermediate phenotypes would define more homogeneous forms of the disorder. In agreement with this hypothesis, we first aimed to assess the familiality of intermediate phenotypes of interest in psychosis and, second, to study their correlation with genetic variability in genes associated with the risk for psychotic disorders.

Familiality of intermediate phenotypes associated with psychosis

The first step is to evaluate to what extent the manifestation of an intermediate phenotype (clinical, cognitive or neurodevelopmental markers) reflects the underlying genetic heterogeneity of the disorder. In this sense, family samples with offspring with the disorder of interest can be used to examine if a phenotype aggregates in all or in a subset of families more than expected by chance. In this respect, we have confirmed the familiality (phenotypic resemblance among family members) of schizotypy and cognitive functions in a sample of families with one offspring with a psychotic disorder. The strength of the familial effect is measured by calculating the family-level residual intraclass correlation coefficient (ICC), which scores from 0 (no aggregation) to 1 (complete familial aggregation). According to our results, schizotypy (ICC=0.30 $p<0.001$) and the cognitive dimension of attention and working memory (AW) (ICC=0.37 $p=0.0004$) significantly aggregate in families affected by schizophrenia or early-onset bipolar disorder, respectively, at a moderate degree. These results are in line with previous studies that revealed schizotypy and cognitive functions as psychosis-related intermediate phenotypes with a moderate heritability (Linney *et al.*, 2003; Knowles *et al.*, 2014; Blokland *et al.*, 2017; Bigdeli *et al.*, 2020).

As described in the introduction (see section 1.2), psychotic disorders show a great heterogeneity both within-subjects (e.g. a particular cognitive dimension might be impaired while another might be intact) and between-subjects (each individual might

exhibit a specific clinical profile, different from that of other patients). If we move the focus from individuals to families, this assumption can be sustained; this is, in a given population, there are some families in which their members may share a similar clinical and subclinical profile and other families in which their members do not resemble each other at all. In this regard, the estimation of the ICC in a sample of families allows us to know the degree of aggregation of a particular phenotype in the global sample; however, it does not help us to interpret the heterogeneity of the phenotype within each family. To overcome this limitation, the present thesis has proposed a strategy that allows moving from a sample-based result (familiality) to a single-family characterization. After confirming the familial nature of schizotypy and the AW dimension, we developed a new method to quantitatively estimate the similarity of these traits among family members (intrafamilial resemblance score, IRS) that can be used to classify families according to their shared level of vulnerability for psychotic disorders. The theoretical basis underlying this strategy is that those families in which their members share similar scores of a familial trait (schizotypy, AW) present a higher genetic component than those families in which their members show discordant values and, therefore, they represent subgroups of families with more homogeneous liability factors for the disorder. Accordingly, this strategy might improve the study of the genetic complexity of psychotic disorders by analysing the clinical heterogeneity pattern observed within families in order to identify more homogeneous forms of disorders.

By using this method, we were able to differentiate families in which all members were similar due to low schizotypy scores of the first-degree relatives (as a proxy of low vulnerability for psychosis) from families with low resemblance because at least one relative presented a high score on schizotypy. These discordant families might be conceptualised as a subgroup that carries a genetic vulnerability for psychotic disorders mediated by common factors with schizotypy, as previous studies suggest (Grant, 2015). In addition, we analysed whether the schizotypy familial aggregation pattern is associated with the presence of another psychotic vulnerability marker such as ridge dissociations, in this case from a neurodevelopmental point of view. We found that relatives with ridge dissociations belonged to discordant families and showed high schizotypy scores, providing evidence that both markers (schizotypy and

ridge dissociations) could be influenced by shared risk factors related to neurodevelopmental processes, which in turn could increase the risk of psychosis. From these results arise the importance of neurodevelopment as a critical period of exposure to different genetic and environmental factors that increase the risk for schizophrenia and other psychotic disorders. As introduced in section 1.5, some of the schizophrenia-associated genes show a greater expression during fetal life (Birnbaum *et al.*, 2014; Birnbaum and Weinberger, 2017) and develop specific roles in early brain development (Fromer *et al.*, 2014). Moreover, there is consistent evidence of environmental factor adversity during neurodevelopment influencing risk for psychosis (Robin M. Murray *et al.*, 2017). Therefore, studies such as ours that contribute to the stratification of patients according to the underlying risk factors might yield insight into disease mechanisms, novel targets for therapeutic interventions and even, prediction and prevention before the onset of the disorder (Weinberger, 2017).

Similarly, in a sample of families with at least one patient with early-onset bipolar disorder, we also differentiated families whose members shared a low performance on attention and working memory (AW) from those whose members showed normal scores. Our results are in line with previous family studies showing the role of AW as bipolar disorder liability markers (Bora, Yucel and Pantelis, 2009b; Volkert *et al.*, 2016; Calafiore, Rossell and Van Rheenen, 2018). Also, they seem to be suggestive about the interest of the AW dimension as a familial liability marker that might be used to distinguish different familial subgroups with a differential genetic loading for attention and working memory deficits.

Based on that the familial aggregation of psychosis is partly explained by genetic risk variants (Agerbo *et al.*, 2015), it may be hypothesized that the different familial patterns observed in relation to schizotypy and AW dimension might be underlyed by genetic differences and thus, that this approach might contribute to dissect the clinical heterogeneity of disorders by identifying forms of disorders with a differential genetic loading for particular phenotypes. Therefore, the next challenge is the inclusion of genetic data in these models. Unfortunately, in the present dissertation, this has not been conducted as larger samples are needed. However, we are already working so that the next studies will be complemented with genetic data in order to

assess whether genetic differences arise between the identified subgroups of families. Nevertheless, despite being hypothetical, it may be interesting to speculate on the genetic differences between the identified subgroups related to the AW dimension, based on the large amount of data available concerning the relationship between common variant genetic liability to psychosis and cognition. We could first hypothesize that the differential patterns related to the AW dimension observed in our sample were due to different genetic variants associated with both psychosis and cognitive function (Toulopoulou *et al.*, 2007). Indeed, it has been suggested that cognitive deficits mediate part of the association between genetic factors and schizophrenia (Toulopoulou *et al.*, 2018); and thus, we could even expect that differences between groups of families reflected distinct causal pathways mediated by genes underlying the AW dimension. On the contrary, other studies have revealed that variance in cognition is substantially independent of the liability to schizophrenia (Toulopoulou *et al.*, 2007; Richards *et al.*, 2020). Accordingly, we could also hypothesize that the different homogeneous subgroups of families detected in our study reflect differences in the genetic loading underlying the AW dimension profile but do not capture different forms of the disorder mediated by specific familial cognitive profiles. In order to make progress on this issue, our group has recently used this approach (familial aggregation + IRS estimation) to stratify an independent sample of families affected with obsessive-compulsive disorder (OCD) according to their level of similarity on cognitive function, and further analyses are planned to assess whether the different identified subgroups of families are mediated by differences in their PRS of schizophrenia and cognition. Moreover, as familial effects may also include the familial environment, another step forward is to extend the familial approach to more complex models that allow testing different phenotypes at the same time as well as the consideration of environmental variants.

In all, while more studies with larger samples are needed, our results illustrate the potential value of combining of the analysis of the pattern of aggregation of intermediate phenotypes in families with an affected offspring with the estimation of the familial resemblance scores (IRS estimation). With such an approach, it is possible to identify more homogeneous subgroups of individuals according to their clinical and subclinical profile, as we hypothesized. Moreover, we also revealed that the study of

the aggregation pattern of a phenotype in relatives might help predict some of the patient's clinical characteristics. Concerning schizotypy, we found that patients of those families with higher scores had more severe disorganised symptoms at the psychotic episode and 1 year later than other patients. This is consistent with previous studies showing the high heritability and familial aggregation of this dimension (Rietkerk *et al.*, 2008; Peralta *et al.*, 2015). Despite studies with larger samples are needed to validate these results, predicting the clinical outcome of patients from the subclinical profile of their relatives is a promising development that may improve the diagnosis and treatment of the disease. In the last years, there has been a growing interest in identifying biomarkers associated with psychosis (Fond *et al.*, 2015); however, due to the clinical heterogeneity and the multiple aetiological factors underlying these disorders, it is highly unlikely that a single biomarker will be able to predict the onset and trajectory of psychotic disorders. In this sense, it is expected that the combination of many biomarkers, including neuroimaging, cognitive, genetic, epigenetic or metabolic data, might be of utility in the clinical practice (Rodrigues-Amorim *et al.*, 2017). Indeed, machine learning techniques have been used to find differential patterns in clinical markers to define more homogeneous forms of a disorder (Schnack, 2019). In this context, further studies should be conducted to assess whether the characterization of the aggregation pattern of a phenotype in relatives could be used, together with other biomarkers, as a potential tool to improve the diagnosis and treatment of psychotic disorders.

Gene-Phenotype correlation

The clinical heterogeneity of psychotic disorders is a reflection of the underlying genetic complexity, as they are caused by multiple combinations of common and rare variants. Moreover, genetic variants that increase the risk for psychotic disorders are pleiotropic, meaning that they are also related to other mental conditions, including major depressive disorder, attention-deficit hyperactivity disorder or autism-spectrum disorder and behavioural traits such as cognitive performance (Hamshere *et al.*, 2013; Lee *et al.*, 2013; Anttila *et al.*, 2016; Witt *et al.*, 2017). As might be expected, the genetic overlap between mental disorders is accompanied by an overlap at the clinical and physiological level. For instance, if we focus on the association between

schizophrenia and autism-spectrum disorders, evidence suggests that they may partially overlap in the clinical, neurobiological, behavioural and cognitive features and that they may have some common etiological roots (Stone and Iguchi, 2011). In this regard, epidemiological and family-based studies have shown a shared genetic liability between both disorders (Larsson *et al.*, 2005; Daniels *et al.*, 2008; Rapoport *et al.*, 2009; Mortensen, Pedersen and Pedersen, 2010; Bevan Jones *et al.*, 2012). Also, GWAS have identified that common genetic variants underlying both schizophrenia and autism-spectrum disorders are particularly enriched in genes involved in the glutamatergic synapse (Purcell *et al.*, 2009; Weiss *et al.*, 2009; Ripke *et al.*, 2014). Glutamatergic synapses are composed of several hundreds of proteins essential for ensuring normal synaptic transmission and plasticity, such as scaffolding proteins, which bring together two or more proteins to facilitate their interaction and functions. In this sense, genetic studies have brought to light the association between genes encoding for scaffolding proteins and the risk for both schizophrenia and autism disorders (Bayés *et al.*, 2011; Chen *et al.*, 2014; Föcking *et al.*, 2014; Fromer *et al.*, 2014; Purcell *et al.*, 2014). Accordingly, we conducted a systematic review in which genetic and molecular data generated across studies was integrated to discuss the relationship of these genetic variants to the clinical and cognitive traits of schizophrenia and autism-spectrum disorders. This review has been included in the present dissertation as it contributes to delivering an updated and comprehensive overview of the genetic landscape underlying psychiatric disorders.

As expected, the review illustrates the complexity and genetic pleiotropy underlying psychiatric disorders. Despite common (SNPs) and rare (SNVs and CNVs) variants were identified in scaffolding genes in both schizophrenia and autism-spectrum disorders, only a few (especially CNV) were detected in both diagnoses. In addition, some of the variants that were associated with a diagnosis of schizophrenia or autism-spectrum disorders seem to be also relevant to the modulation of cognitive performance in both patients and healthy individuals. This lack of specificity may be explained in terms of the pleiotropic nature of scaffolding genes. Variants in different scaffolding genes, either at the allelic or the gene level, may deregulate the glutamatergic synapse's homeostasis, and this is finally expressed as features observed in different neurodevelopment disorders. These results again bring to the

discussion the usefulness (or the lack of usefulness) of establishing categorical diagnostic boundaries in genetic research and highlight the need to consider the clinical heterogeneity within each diagnosis in order to establish much more precise gene-phenotype relationships.

In this regard, the present thesis has contributed to the understanding of the gene-phenotype correlation by assessing the relationship between genetic variants in genes encoding for different synapse function and regulatory proteins with intermediate phenotypes related to psychosis, including cognitive function and schizotypy. These analyses were conducted in a sample of families affected with psychotic disorders and in a sample of healthy individuals from the general population. As it has been already introduced (see section 2.2), intermediate phenotypes are quantifiable measures related to specific neurobiological functions present in the general population, although at a lower rate than in patients or their relatives. Due to this circumstance, the study of the effects of risk genotypes in healthy individuals is a common practice to avoid the potential contamination of signal from non-genetic and/or illness-related factors, including treatment, symptoms or environmental issues that make it difficult to interpret results in patients alone.

Regarding the relation between schizophrenia-associated genetic variants and cognitive functions, we found significant associations between *DAOA* and *AKT1* genes with executive function and attention, respectively. About the *DAOA* gene, we particularly found that a haplotype at this gene was undertransmitted to patients with schizophrenia and to healthy relatives, whereas another haplotype was associated with worse scores in an executive function-related task in subjects with the disorder but not in healthy relatives. These results suggest that the *DAOA* gene might contribute both to the risk for schizophrenia and to the modulation of executive function, which is in accordance with previous studies showing the association between this gene and schizophrenia (Liu *et al.*, 2019) and cognitive performance (Goldberg *et al.*, 2006; Donohoe *et al.*, 2007; Opgen-Rhein *et al.*, 2008). Interestingly, several SNPs within this gene have been associated with a protective effect in psychotic disorders (Ma *et al.*, 2006; Bass *et al.*, 2009), maybe by regulating brain functional and/or anatomical intermediate phenotypes (Haznedar *et al.*, 2005; Schultz

et al., 2011; Woodward and Heckers, 2015). Although the reported association of the *DAOA* gene with executive functions seems to be dependent on the diagnosis, further studies should be conducted to investigate the relationship between this gene and both phenotypes. Moreover, in order to be able to properly interpret our results, the role that this gene play in brain function must be reminded. In this sense, the *DAOA* gene encodes the protein DAOA, which activates D-amino acid oxidase (DAAO) in the brain, an enzyme that oxidizes D-serine, an important co-agonist for the N-methyl-D-Aspartate receptor (NMDAR) (Chumakov *et al.*, 2002). As explained in the introduction, subjects with a diagnosis of schizophrenia have increased DAOA expression in the pre-frontal cortex (Korostishevsky *et al.*, 2004) and lower D-serine levels in serum (Hashimoto *et al.*, 2003) and cerebrospinal fluid (Hashimoto *et al.*, 2005) than healthy controls (see **Box 3**). Taking this data into account, it could be suggested that genetic variability might contribute to the overexpression of *DAOA*, which in turn could result in NMDR hypofunction by decreasing D-serine concentration.

Concerning the *AKT1* gene, an effect of this gene on sustained attention was detected in a non-clinical sample, as previous studies had also described (Tan *et al.*, 2008; van Winkel *et al.*, 2011; Ohi *et al.*, 2013). Particularly, we observed that individuals with two copies of the haplotype C-A (rs2494732–rs1130233) performed better in this cognitive dimension. The *AKT1* gene encodes the serine/threonine kinase Akt1, involved in modulating synaptic dopaminergic transmission systems, where it is a key signalling intermediate downstream of dopamine receptor D2 (DRD2) (Scheid and Woodgett, 2001, 2003; Beaulieu *et al.*, 2005). Thus, it seems plausible that genetic variation within this gene might modulate cognition through the regulation of dopaminergic neurotransmission.

Despite few studies suggesting that genetic variation underlying cognition is independent of the genetic variation that increases the risk for schizophrenia (Toulopoulou *et al.*, 2007; Richards *et al.*, 2020), other studies tend to show a significant negative correlation between cognitive performance and a schizophrenia diagnosis, as genetic loading for lower cognitive scores is associated with greater risk for the disorder (Bulik-Sullivan *et al.*, 2015; Smeland *et al.*, 2017; Trampush *et al.*,

2017; Savage *et al.*, 2018). In this context, while *DAOA* and *AKT1* genes have been associated with schizophrenia (Schwab *et al.*, 2005; Bass *et al.*, 2009) and cognitive functions (Jansen *et al.*, 2009; Pietiläinen *et al.*, 2009) in several candidate-gene based studies, they have not been implicated in any GWAS, either regarding psychosis or cognition. The absence of these genes from the list of results coming from large GWAS should give us pause for thought. A plausible explanation could be that the effect of these genes on these traits, which are highly heterogeneous, is diluted within the phenotypic diversity present in large samples and, thus, that the detection of these genes' effect is highly conditioned to the reduction of the clinical heterogeneity of the sample (Van Rheenen *et al.*, 2017). In this sense, future GWAS might try to address this issue by prioritizing a better phenotype characterization and stablishing more precise inclusion criteria, as different studies have suggested (Peralta and Cuesta, 2000; MacRae and Vasan, 2011; Manchia *et al.*, 2013; Traylor, Markus and Lewis, 2015; Kulminski *et al.*, 2016; Power *et al.*, 2017). Accordingly, if we want to advance in the disentangling of the neurobiological basis underlying psychosis and cognition, traditional candidate gene studies, such as the one included in the present thesis, should be conducted to follow-up GWAS implicated genetic variants within deeply phenotyped individuals from which greater insights on the clinical and functional relevance of these variants may be gained.

This is indeed what we have done with the *ZNF804A* gene, in which a main effect of the polymorphism rs1344706 on the interpersonal dimension of schizotypy was detected. Specifically, AA homozygotes showed the highest Interpersonal schizotypal scores. Our results add evidence on this SNP increasing psychosis vulnerability, equally to different schizophrenia GWAS (O'Donovan *et al.*, 2008; Riley *et al.*, 2010; Williams *et al.*, 2011; Falola *et al.*, 2017; Pardiñas *et al.*, 2018) and diverse studies based on schizotypy measurements suggest (Yasuda *et al.*, 2011; Stefanis *et al.*, 2013; Meller *et al.*, 2019).

Given that the association between rs1344706 in *ZNF804A* and schizophrenia is reproducible, its possible role in the pathogenesis of the disorder has been intensively studied during the last years (Chang, Xiao and Li, 2017). In this sense, it has been suggested that *ZNF804A* plays a role in the regulation of brain development and the

functioning of synaptic plasticity (Penzes *et al.*, 2011; Hill and Bray, 2012; Hess and Glatt, 2014; Deans *et al.*, 2016). Expression studies have shown that the A allele of rs1344706 is associated with reduced expression of *ZNF804A* RNA in patients with a diagnose of schizophrenia, indicating its decreased expression as a likely risk for the disorder (Hill and Bray, 2012; Tao *et al.*, 2014). Indeed, reduced ZNF804A expression in neurons has resulted in aberrant neurite growth and loss of dendritic spine density (Penzes *et al.*, 2011; Konopaske *et al.*, 2014) which is also consistent with the clinical observations in the brains of patients with a diagnose of schizophrenia. Taken together, although further studies are needed, it seems that the path to understanding how changes in the *ZNF804A* sequence can impact clinical and subclinical phenotypes has started to be drawn.

As introduced in section 2.2.1, schizotypy is linked to clinical risk for psychosis (Barrantes-Vidal, Grant and Kwapil, 2015). However, while several studies have reported an effect of the *ZNF804A* gene on schizophrenia and schizotypy, it is unclear whether and to what extent schizotypal traits overlap genetically with risk for schizophrenia. In contrast to a growing number of studies reporting an association between PRS for schizophrenia and cognition (Trampush *et al.*, 2017; Shafee *et al.*, 2018; Habtewold *et al.*, 2020), only a few have used genome-wide approaches to test the relation schizophrenia-associated genetic risk, and schizotypal traits and they have produced inconsistent results. While two studies have found associations between schizotypy and schizophrenia risk genes (Fanous *et al.*, 2007; Ortega-Alonso *et al.*, 2017), another did not find correlations of schizophrenia PRS with either global scores of schizotypy or specific dimension scores (Nenadić *et al.*, 2020). Although more studies are needed to shed light on this picture, we can try to draw a few scenarios to explain this phenomenon. A first scenario would be that schizotypy was not related to genetic psychosis risk. While possible, this seems highly unlikely given the accumulating evidence showing that schizotypal traits are more frequent in relatives of patients with schizophrenia and prodromal states. Moreover, high schizotypy measurements have been associated with brain functional and structural alterations and lower cognitive performance (Ettinger *et al.*, 2015; Siddi, Petretto and Preti, 2017; Wang *et al.*, 2020; Kozhuharova *et al.*, 2021). A second scenario could be that only part of the genes and pathways underlying schizophrenia would

modulate the expression of schizotypy. As an example, the PRS of schizophrenia is not associated with attention and brain activity in non-clinical individuals; however a particular PRS estimated with a subset of SNPs related to the glutamatergic synapse is (Rampino *et al.*, 2017). Thus, a similar picture may be ruling the relationship between schizophrenia and schizotypy. Indeed, another study has shown that the structural brain changes associated with schizotypy are related to glutamatergic levels, supporting the hypothesis that variation in the glutamatergic function may lead to structural, and consequently, functional changes associated with the expression of psychosis (Modinos *et al.*, 2017). A third scenario requires considering schizotypy as a complex trait that emerges from gene-environment interactions (MacDonald *et al.*, 2001; Ericson *et al.*, 2011). Thus, it may be possible that the gene-schizotypy correlation is dependant on the presence of certain environmental factors. In this respect, Hatzimanolis et al. (2018) found a relationship between schizotypal traits and schizophrenia PRS, in a male sample, but only under particular environmental conditions (stress).

In all, the observed results support our hypothesis; the use of intermediate phenotypes has proven to be an adequate strategy to identify genetic variants that increase the risk of psychosis. Despite this apparent success, there are studies questioning the validity of the intermediate phenotypes to improve the gene discovery of schizophrenia by arguing that intermediate phenotypes are equally complex as the disorder (Flint, Timpson and Munafò, 2014; Greenwood *et al.*, 2019). Thus, future studies should focus on understanding the heterogeneity observed within these intermediate phenotypes. In this regard, and according to the value that the analysis of familial aggregation pattern's of different phenotypes has shown in identifying more homogeneous forms of complex disorders, its implementation could be helpful to disentangle the heterogeneity of intermediate phenotypes.

Gene x Cannabis interaction

Both genetic and environmental factors modulate complex phenotypes such as cognition and schizotypy. The study mentioned above that finds that schizotypy levels and schizophrenia PRS are associated only under stress conditions is a perfect

example to illustrate this (Hatzimanolis *et al.*, 2018). Accordingly, gene-environment interaction analyses are needed to assess whether environmental risk factors mediate the relationship between genetic factors and phenotypes. With this aim in mind, in the present dissertation it has been analysed whether cannabis use modulates the effect of the two identified gene-phenotype associations (*AKT1*-cognitive performance and *ZNF804A*-schizotypy).

On the one hand, the GxE interaction analyses found that lifetime cannabis use might act as a modifier of the association between the rs1344706 (*ZNF804A*) and schizotypy (specifically, the cognitive-perceptual dimension). Among cannabis users, homozygous for the risk allele (AA) showed the highest schizotypy scores, following a dose-response relationship. Thus, we observed that schizotypy scores increased as the frequency of cannabis use did. Our results are in line with previous studies reporting a dose-response relationship between the frequency of cannabis use and the risk for psychosis (Marconi *et al.*, 2016; Wainberg *et al.*, 2021) and suggest that the individual genetic background could mediate this relationship. In this regard, although this is the first study to assess the interaction between the *ZNF804A* gene and cannabis use frequency on schizotypy, other studies have already reported this interaction with other genes, including *AKT1* or *COMT* (Caspi *et al.*, 2005; Van Winkel *et al.*, 2011).

On the other hand, our results concerning the *AKT1*–cannabis interaction on cognition were in agreement with those reported by Van Winkel et al. (2011). Despite finding a significant interplay between this gene and cannabis use on attention measures in patients with a diagnosis of schizophrenia, they did not find such an interaction in healthy individuals. Altough other studies have also identified the interaction between this gene and cannabis use on cognitive performance in subjects affected with schizophrenia (Henquet *et al.*, 2006; González-Pinto *et al.*, 2016), data on healthy individuals are scarce and further interaction analyses should be conducted to better understand the relationship between *AKT1* variability and cannabis use on cognition.

Regarding the relation between the use of cannabis and the *ZNF804A* gene on the risk for psychosis, there is evidence coming from experimental and longitudinal

studies suggesting that cannabis interacts with genetic and environmental liability factors leading to psychotic symptoms (van Os *et al.*, 2002; Verdoux *et al.*, 2003; D'Souza *et al.*, 2005; Henquet *et al.*, 2005; Kahn *et al.*, 2011; Decoster *et al.*, 2012; Radhakrishnan, Wilkinson and D'Souza, 2014; van Winkel and GROUP Investigators, 2015; Wainberg *et al.*, 2021). Nevertheless, it is also well known that there is a genetic background for the likelihood of using cannabis (Kendler *et al.*, 2008) and that this overlaps with the genetic risk of psychotic disorders (Power *et al.*, 2014; Gage *et al.*, 2017; Verweij *et al.*, 2017). This phenomenon, called gene-environment correlation, means that individuals at high genetic risk for schizophrenia are more likely to use cannabis. According to this, the genotype increases both the propensity to develop psychosis and, although at a very low impact, the likelihood of being exposed to cannabis (genetic risk for schizophrenia explains less than 1% of the variance in cannabis use; Verweij *et al.*, 2017). Therefore, although it is unlikely that gene-environment correlation fully explains the association between cannabis use and psychosis, it is important to take this possibility in mind when interpreting our results. In our case, despite the modest sample size, specific gene–environment correlations between *ZNF804A* or *AKT1* and cannabis use were not detected, ruling out the effect of the genes on cannabis use in our sample.

It is also appropriate to note that the GxE studies performed in this thesis have tested the interaction between one candidate gene and one environmental factor. However, in accordance with the complex nature of psychiatric disorders, the risk for psychotic disorders is explained by many variants in multiple genes and the exposure to several environmental factors, including cannabis use but also many others, such as urban birth, the season of birth, obstetric complications, childhood adversity or abuse (see sections 1.5 and 1.6). Given the complex aetiology of psychotic disorders, the GxE research might be enhanced with the use of polygenic risk score (PRS) and/or an environmental risk score (ERS) for psychosis. For example, a recent study that examined the interaction between genetic liability and environmental exposure as a risk of psychosis using PRS and ERS showed that those subjects with higher exposure to genetic and environmental risk factors are more susceptible to develop a first-episode psychotic than those only showing a high genomic or environmental load risk (Mas *et al.*, 2020).

Remarkably, unlike genetics, environmental factors can be modified selectively among those at high genetic risk, providing the opportunity to use information about the environment to develop more targeted and effective public health prevention and intervention programming (Bratman *et al.*, 2019; Paquin *et al.*, 2021). Hence, GxE analyses should gain importance in the future. In this regard, despite our GxE analyses are modest in comparison with large studies assessing the interaction between PRS and ERS, they have contributed to understanding the relationship between cannabis use and the risk for psychosis, proving that GxE studies can shed a lot of light on the investigation of the aetiological basis of psychosis.

Overall limitations of the presented work

The different studies conducted in this thesis present some limitations. Although they have already been exposed throughout the discussion, the most relevant are described below.

The main limitation of all the presented works is the moderate sample sizes and the lack of replication samples. However, the difficulty of sampling families must be taken into consideration. Moreover, as discussed above, the balance between a large sample and a well-phenotyped sample is challenging and the limited sample sizes in our studies were detrimental to achieving a better characterisation of diverse intermediate phenotypes.

Second, psychotic disorders are caused by many genetic risk variants that interact with different environmental risk factors. Thus, it is evident that the study of particular genetic or environmental variants in the risk for psychosis must be interpreted with caution as the detected genetic and environmental effects on this risk are small. In this regard, our studies assessing the impact of particular genes and/or one environmental risk factor on psychosis present a constraint in front studies evaluating the effect of thousands of variants by using PRS and ERS, leading to more global conclusions about the ethiopathogeny of psychosis. As a counterpart, our studies contribute to disentangling the complexity underlying psychotic disorders by drawing specific correlations between particular genetic variants, cannabis use and

intermediate phenotypes. Moreover, both the genes and the use of cannabis were selected based on previous findings, as explained in the introduction (see **Box 3** and **Figure 10**).

Another limitation is the lack of control for the pharmacological treatment effects of the individuals included in the different studies. As pharmacological treatment was not always available, statistical analyses were not adjusted for treatment type or duration. This may be of certain importance on those studies where the cognitive performance was the dependant variable as it is not clear to which extent different pharmacological treatments could impact on cognition (Baldez *et al.*, 2021), although several pharmacological trials have reported that the action of antipsychotic drugs on neurocognitive improvement ranges from minor to neutral and that there are no differences between treatments, including typical and atypical antipsychotic drugs (Keefe *et al.*, 2007; Millan *et al.*, 2012). Moreover, the presence of confounding factors inherent to psychosis such as medication was ruled out in those studies conducted in non-clinical samples. Finally, this issue has been intended to address in the two studies assessing the familiality of schizotypy and cognition performance by means of conducting the aggregation analyses in the total sample and in a subset of only healthy relatives. The degree of aggregation of the different phenotypes was the same in both samples, suggesting that the observed effect was independent of disease status (and, therefore, independent of treatment).

The last potential constraint refers to the lack of available data concerning the cannabis use evaluation, including the age of initiation, the type of cannabis used, the amount consumed and the last time of consumption, which therefore reduces the precision of our measure. Nevertheless, recent findings suggest that one of the strongest predictors of whether any given individual would have a psychotic disorder or not is the cannabis use frequency (Di Forti, Quattrone, Tom P. Freeman, *et al.*, 2019). In this sense, our analyses tested a possible dose-effect relationship of cannabis use on the evaluated measures as frequency data were available.

Final remark

As a final remark, I would like to retrieve the importance of the psychosis continuum model as the framework in which the present thesis has intended to analyse schizophrenia and other psychotic disorders. As explained along this dissertation, data from clinical, epidemiological and genetic studies suggest that the different psychosis expressions are distributed in a continuous way in the general population, from subclinical manifestations to the extreme symptoms observed in people diagnosed with a psychotic disorder such as schizophrenia or bipolar disorder. This distribution, in turn, might mirror a continuous distribution of the vulnerability factors, both genetic and environmental, across the population, meaning that the same factors that influence the risk of psychotic disorders also influence the prevalence of subclinical symptoms in the general population (van Os and Linscott, 2012; DeRosse and Karlsgodt, 2015). Based on this model, to understand the clinical heterogeneity and genetic complexity underlying psychotic disorders, we must interpret these phenotypes as continuous traits across a spectrum in the population. In this sense, far from understanding psychosis as a categorical construct, this thesis has attempted to study such a broad phenotype from a dimensional perspective, taking into account the whole population and different clinical and subclinical manifestations expressed along this continuum. Thus, we have worked not only with this end of the population that has a psychiatric diagnosis, but also with subjects belonging to the intermediate part of the distribution who do not have any psychiatric diagnosis but are considered high-risk individuals, either because they share genetic (healthy relatives of individuals with psychosis) or environmental factors (cannabis use) that increase the risk for psychosis.

The findings reported in the articles compiled in the present thesis have proved that the use of family-based samples, including a psychotic patient and his/her healthy relatives, might improve our ability to find familial vulnerability markers or intermediate phenotypes for psychosis, which in turn can be used to identify more homogeneous forms of psychotic disorders and thus facilitate research into the genetic risk variants underlying these forms. Moreover, family-based samples have also facilitated the identification of psychosis-associated genetic risk variants. Regarding the use of non-

clinical samples, they have been helpful to investigate the interaction between particular schizophrenia-associated risk genes and the use of cannabis in individuals with high variability in two intermediate phenotypes associated with psychosis, such as schizotypy and cognitive function.

Finally, despite the last advances in the comprehension of the genetic aetiology of psychotic disorders the identification of the involved genetic factors has still a long way to go. With few exceptions involving rare genetic variants, we are still far from predicting clinical phenotypes from the measurement of the genetic liability and personalized medicine based on the different genetic background of each individual is still seen as a distant clinical practice. Thus, it is necessary to continue making efforts towards understanding the etiopathogenic basis of psychotic disorders, taking into account not only the genetic but also the environmental factors and the interaction of the two. It is through research that we can build knowledge that will ultimately lead to an improvement in the quality of life of those affected by psychotic disorders. In this regard, the present dissertation has intended to provide our grain of sand to the collective construction of knowledge on the aetiology of psychosis by means of using different strategies that have proven to contribute to elucidating the heterogeneity underlying these disorders, which in turn might lead to an improvement of the identification of the underlying causal genetic variants.

CONCLUSIONS

Specific conclusions derived from the present dissertation are developed below:

I. The study of the familiality of intermediate phenotypes of interest in psychosis (schizotypy, neurodevelopmental markers, cognitive performance) and the subsequent estimation of the intrafamilial resemblance between family members on them enables the identification of more homogeneous forms of psychotic disorders.

    a. Schizotypy is a familial phenotype and families can be classified based on the schizotypy resemblance between family members. Healthy relatives with higher schizotypy levels present a higher prevalence of ridge dissociations, another psychosis liability marker. The schizotypy aggregation pattern in relatives might be used to predict patients' clinical characteristics.

    b. In our sample, the cognitive dimension of attention and working memory (AW) has proven to be a putative familial liability marker for early-onset bipolar disorder. It can be potentially used to indentify subgroups of families with different genetic loading.

II. Genes involved in synaptic plasticity are associated with the risk for psychosis, as measured with the expression of intermediate phenotypes, in clinical and non-clinical samples.

    a. Genetic variability at the *DAOA* gene modulates executive function performance in patients with psychosis, while *AKT1* modulates attentional responses in non-clinical individuals.

    b. Genetic variability at the *ZNF804A* gene is associated with the interpersonal dimension of schizotypy in non-clinical individuals.

c. Genetic variation within scaffolding genes is pleiotropic and contribute to the shared genetic liability across psychiatric disorders, including schizophrenia and autism-spectrum disorders.

III. In our sample, the use of cannabis modulates the effect that genetic variation within the *ZNF804A* gene has on the risk for psychosis, measured with the expression of schizotypy. In contrast, the association between the *AKT1* gene and attention measures is not modified by the exposure to cannabis.

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