Title: Role of neurotrophins in depressive symptoms and executive function: Association analysis of NRN1 gene and its interaction with BDNF gene in a non-clinical sample

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ABSTRACT

Background: Neuritin-1 (*NRN1*) is a neurotrophic factor involved in synaptic plasticity that has been associated with schizophrenia, depressive disorders and cognitive performance. Considering that the study of genotype-phenotype relationship in healthy individuals is a useful framework to investigate the etiology of brain dysfunctions that underlie mental disorders, we aimed to study in a general population sample, whether *NRN1* gene variability is contributing to: i) the psychopathological profile, ii) the executive function performance. We also aimed to test whether these associations are modulated by *BDNF* gene.

Methods: The sample comprised 410 subjects from the general population who filled in the self-reported Brief Symptom Inventory (BSI) and were assessed for cognitive executive performance using 3 neuropsychological tests including Verbal Fluency, Wisconsin Card Sorting Test (WCST) and Letter-Number subscale (WAIS-III). Genotyping analyses included 9 SNPs in *NRN1* and one in *BDNF* (Val66Met).

Results: i) GG homozygotes of rs1475157-*NRN1* showed higher scores on BSI depressive dimension and total scores (β=0.62 p=0.00036, β=2.51 p=0.00033, respectively). ii) A linear trend was detected between GG genotype of rs1475157 and a worse cognitive performance in: Phonemic Fluency: β=-1.66 p=0.086; WCST total-correct response: β=-4.5 p=0.029. iii) Interaction between rs1475157-*NRN1* and Val66Met-*BDNF* was found to modulate depressive symptoms (p=0.001) and phonemic fluency (p=0.033).

Discussion: Our results suggest that *NRN1* variability has a role in the presence of depressive symptoms and in modulating executive function performance. These effects seem to be modulated by *BDNF*, which supports a gene-gene interaction effect between both neurotrophic factors in a general population sample.

KEYWORDS

Depressive symptoms, cognitive performance, *NRN1*, *BDNF*, gene-gene interaction

INTRODUCTION

Brain development is a well-organized dynamic process which efficiency is essential for the correct functioning of the whole brain. Both, genetic and environmental inputs are involved in a normal brain development, and disruption of either can fundamentally alter neural outcomes. Accordingly, deviances in neurodevelopmental processes are thought to contribute to the etiology of many psychiatric disorders that manifest throughout the entire lifespan. Increasing evidence suggest that Neurotrophic factors (also called Neurotrophins, *NTFs*) are important regulators of neural survival, growth, development, function, and plasticity (Huang and Reichardt 2001). In this sense, an inadequate neurotrophic support in the brain could lead to an inappropriate cortical circuitry and synaptic transmission in the developing brain, which could translate into a reduced brain's ability to make adaptive changes (Angelucci et al. 2005). This lack of plasticity, in turn, could be underlying the cognitive functioning variability in healthy individuals and the brain alterations related to the development of mental disorders.

Variability of brain plasticity, from health to disease status, is thought to result from complex interactions between genetic factors following a polygenic inheritance pattern in which multiple genes with small effects are involved. In addition, recent molecular genetic studies indicate empirical evidences of the existence of some shared genetic roots between several psychiatric disorders such as Schizophrenia (SZ) or Major Depressive Disorder (MDD) (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013); which adds to the consideration of common pathophysiological mechanisms among these disorders. This view is reinforced by the fact that these disorders show impairments across similar domains including attention, memory, cognitive control and Executive Function (EF) (Chamberlain and Sahakian 2004 ; Blarch and Sheffield 2014), even though quantitative differences may exist. In particular, EF represents cognitive processes, including the ability to sustain and shift attention, inhibit pre-potent responses, hold information in working memory, and plan responses (Pennington and Ozonoff 1996), and there is strong argument that EF is particularly compromised in both SZ and MDD (Elliott 2003).

Due to the important role that neurotrophins play along the neurodevelopment NTFs such as Neuritin-1 (*NRN1*) or Brain-Derived Neurotrophic Factor (*BDNF*) are considered as putative candidate genes for psychiatric diseases. Neuritin-1 gene, also called candidate plasticity gene 15 (CPG15), encodes a small highly conserved protein attached to the extracellular neuronal membrane by a glycosylphosphatidylinositol link and operates as an intercellular signal

between neighbouring neurons (Naeve et al. 1997). As reviewed by Zhou and Zhou (2014), *NRN1* is involved in neurodevelopment and synaptic plasticity, and also in promoting processes such as dendritic and axonal growth, neurite outgrowth, neuronal migration, and the maturation of synapses. Furthermore, the expression of *NRN1* gene has been reported to be regulated by *BDNF* (Naeve et al. 1997), which promotes the differentiation and growth of developing neurons in central and peripheral nervous systems (Buckley et al. 2007).

NRN1 gene has already been involved in the risk for mental disorders and associated phenotypes. On the one hand, previous studies have reported the effect of *NRN1* polymorphic variation on the risk for developing SZ and on general cognitive performance (Chandler et al. 2010; Fatjó-Vilas et al. 2016). On the other hand, from animal model based studies, there is evidence of *NRN1* relationship with depressive symptoms. First, Neuritin knockdown results in depressive behaviors (Son et al. 2012). Second, electroconvulsive therapy, one of the most robust gene inducer among all antidepressant treatments (Segi-Nishida 2011), induces changes in both *NRN1* and *BDNF* expression (Dyrvig M et al 2014; Park HG et al 2014). Third, fluoxetine increases the level of *NRN1* and *BDNF* specifically in the prefrontal cortex, hippocampus and dentate gyrus (Alme et al. 2007), which suggest that antidepressant treatment promotes gene expression responses linked to *NTFs* signaling and synaptic plasticity.

Psychiatric research has been mainly focused on subjects affected by the severe form of the disorders; however, studying subjects presenting attenuated symptoms, without reaching the clinical threshold, may also shed light on the etiology of mental disorders. In this sense, for example, epidemiological studies have reported that depressive symptoms are frequent in the general population, varying between 2.1% and 7.6% (Regier et al. 1988; Blazer et al. 1994). Or, a recent meta-analysis reported that the median lifetime prevalence of Psychotic experiences (PE) was 7.2% (Linscott RJ 2013). These data suggest that there is a continuous distribution of symptoms in the general population where individuals differ in the frequency or intensity of the experience of these symptoms, supporting the notion that both clinical and subclinical symptoms share some of the risk (Verdoux and van Os, 2002). Thus, the study of the factors and mechanisms underlying psychiatric symptoms in non-clinical samples contributes to the understanding of the severe expression of these phenotypes and present the advantage of obtaining results not biased by treatment or the illness itself.

Considering all mentioned above, the understanding of the role of *NRN1* gene may contribute to explain the neuroplasticity mechanisms underlying the qualitative and intensity pattern of

different psychopathological profiles. Accordingly, our study seeks to investigate in a general population sample: i) whether *NRN1* gene variability contributes to the psychopathological profile, with special interest in the dimensions previously related to *NRN1* gene (i.e. depressive and psychotic), ii) the implication of *NRN1* gene in the executive function performance, iii) whether the association between either *NRN1*-psychopathological profile or *NRN1*-cognitive performance is moderated by *BDNF* gene.

METHODS

Sample description

Adult healthy Spanish individuals from the general population were recruited from the campus of Jaume I University in Castelló (Spain).

Exclusion criteria were the presence of any major medical illness affecting brain function, current substance abuse (alcohol or illicit drugs), neurological conditions, history of head injury and personal history of psychiatric medical treatment. These areas were screened by means of a short interview designed *ad hoc* for this study. In addition, participants were required to describe themselves as being of Spanish (Caucasian) ancestry to reduce the possibility of confounding by population stratification (Freedman et al. 2004).

Ethical approval was obtained from local research ethics committees. All participants provided written informed consent before inclusion in the study.

Measurements

All interviews were carried out by trained psychologists.

- ***Brief Symptom Inventory***

All participants filled in the Brief Symptom Inventory (BSI), which is a self-administered scale that provides information for a wide range of symptoms of psychological distress and mental disorders (Derogatis and Melisaratos 1983)~~(Derogatis and Melisaratos 1983)(Derogatis and Melisaratos 1983)(Derogatis and Melisaratos 1983)(Derogatis and Melisaratos 1983)(Derogatis and Melisaratos 1983)~~ in the last 30 days. We used the Spanish validated version of the BSI, which includes 46 items grouped into six dimensions: depression, anxiety, paranoid ideation, obsession-compulsion, somatization and hostility (Ruipérez et al. 2001). The questionnaire was conceived to measure psychiatric symptoms from a dimensional perspective and designed to be used both in clinical and non-clinical population. Each item of the BSI is rated on a 5-point scale of distress ranging from ‘‘not at all’’ (1) to ‘‘extremely’’ (5). As an example, *Paranoid ideation dimension* refers to being susceptible, full of mistrust or with fear of loss of autonomy, among others, and *Depression Dimension* includes signs and symptoms of the clinical syndrome of depression such as dysphoric affect, loss of interest in life activities, or loss of vital energy.

A continuous weighted score of each symptom subscale was used in the analyses (e.g. sum of scores on the depression items divided by number of items filled in).

- ***Cognitive assessment***

Cognitive executive function was assessed using a battery of 3 standardized neuropsychological tests, which have been shown to be sensitive to frontal/prefrontal dysfunction (Lezak et al. 2004): Verbal Fluency (Spreen, O., & Benton 1977), Wisconsin Card Sorting Test (WCST, Heaton 1981)) and Letter-Number subscale of Wechsler Adult Intelligence Scale (WAIS-III, Wechsler 1997). From these tests, 5 outcome variables were selected: 1) number of animals named in one minute (Semantic Fluency), 2) number of words starting with letter P named in one minute (Phonemic Fluency), 3) Number of perseverative errors (WCST), 4) Number of correct responses (WCST), 5) total score on Letter-Number subscale (WAIS-III).
Additionally, the Intellectual quotient (IQ) was assessed using the Block Design and Vocabulary or Information subtests of the WAIS-III, in accordance with the method suggested by Sattler (2001).

Molecular Analysis

Genomic DNA was extracted from buccal mucosa using standard methods: the Real Extraction DNA Kit (Durviz S.L.U., Valencia, Spain) or the Buccal Amp DNA Extraction Kit (Epicentre® Biotechnologies, Madison, WI).

Coverage of *NRN1* genomic sequence and ~10kb upstream and downstream was achieved by including 9 tag SNPs. The optimal set of SNPs that contained maximum information about surrounding variants was selected by using SYSNPs (http://www.sysnps.org/) with a minor allele frequency (MAF) >5%, using pairwise option tagger (threshold of r^2=0.8). The SNPs included in Chandler et al (2010) study were also considered. The functional SNP rs6265 of the *BDNF* gene was also genotyped. For this polymorphism, the A allele encodes for the aminoacid methionine (Met) and the G allele encodes for valine (Val). In subsequent analyses, individuals with Val/Met or Met/Met genotypes were combined (Met carriers) and compared with individuals with the Val/ Val genotype. See Table 2 for SNPs details.

Genotyping was performed using a fluorescence-based allelic discrimination procedure (Applied Biosystems Taqman 5'-exonuclease assays). Standard conditions were used. The genotyping call rate for all SNPs was higher than 94.2%. After randomly re-genotyping the 10% of the sample, the 100% of genotyping results were confirmed.

All SNPs were in Hardy-Weinberg equilibrium.

Statistical analysis

Genotypic association analyses were undertaken between *NRN1* SNPs and each BSI dimension using linear regression function in PLINK (Purcell et al. 2007), including age and gender as covariates. We also explored the data under the assumptions of dominant (major homozygotes versus heterozygotes plus minor homozygotes) or recessive (major homozygotes plus heterozygotes versus minor homozygotes) models of inheritance. These analyses were corrected for multiple testing by using PLINK's max (T) permutation procedure with 1000 iterations.

Based on the significant results of genotypic association analysis, the association between the *NRN1* rs14751157 (GG vs A carriers) and cognitive performance was analyzed by means of linear regression (SPSS 21.0; IBM, New York, U.S.A). Years of education and gender were included as covariates. The relationship between cognitive performance and BSI depressive/total scores was also tested using linear regression, adjusted by years of education and gender. Moreover, interaction between *NRN1* rs1475157 (GG vs A carriers) and the *BDNF* rs6265 (Val/Val vs Met carriers) polymorphisms on depressive dimension and phonemic fluency/WCST correct responses was explored by means of two-way interaction effects with linear regression model. In each of these linear regression analyses Bonferroni correction was applied.

Statistical power estimations were conducted by using G*Power 3.1.7 (Faul et al., 2009). In our sample we had a sufficient power (0.80) to detect a range of effect size (d) of 0.32-0.70 between the two main genotypes of *NRN1* SNPS. Specifically, for rs1475157 (GG vs A carriers) the effect size was d=0.66, which, as an example, corresponds to 0.71 points on BSI depressive dimension scores (Cohen 1988).

RESULTS

· Sample description

The sample was composed of 410 subjects from the general population: 44.2% of males, mean age at interview (sd)= 22.09 (3.4). At the assessment, 77% of the participants were university students. In terms of education, 2.51% of individuals had completed elementary school, 92.46% had completed high school and 5.03% had received a university education (mean years of education(sd)= 13.5 (1.7), mean IQ (sd)=99.16 (11.64)).

In relation to the psychopathological status measured by the BSI, in the current sample between 15-20% of the individuals reported that "extremely" experienced at least one item of the scale (Table 1).

With regard to executive function evaluation, mean(sd) scores of the tests were as follows: i) Phonemic fluency=16.64(4.1); Semantic fluency=22.28(5.1); Perseverative Errors WCST=8.44(8.01); Total correct response=69.98(8.88); Letters-Numbers=9.32(2.52).

Table 2 shows the genotype distribution for *NRN1* and *BDNF* polymorphisms in the sample. In this sense, the observed genotypic frequencies were similar to those described by 1000 Genomes Project.

Table 1. Data on BSI dimensions and total scores (n=410)

BSI dimensions	Mean score (sd)*	% individuals that reported that "extremely" experienced at least one item of the subscale
BSI _depression	1.83 (0.74)	22.46
BSI_anxiety	1.28 (0.39)	16.60
BSI_somatization	1.60 (0.56)	21.49
BSI_hostility	1.38 (0.49)	17.63
BSI_obsession	1.73 (0.60)	22.22
BSI_paranoia	1.72 (0.61)	22.46
BSI_total	9.57 (2.72)	22.46

(*) weighted scores

Table 2. Information on *NRN1* and *BDNF* SNPs included in this study. The table includes the dbSNP number, the genomic and gene position and the alleles of each SNP (UCSC Genome Browser on Human Mar. 2006 Assembly (hg18), http://genome.ucsc.edu/cgi-bin/hgTracks). Observed genotypic and allelic frequencies are also given.

SNP	Chr	Chr Position	Gene position	Alleles[a]	MAF_{1000G}[b]	MAF_{sample}[c]	Genotype Frequency (%)		
NRN1 gene									
rs2208870	6	5992490	Intergenic	A/G	0.332	0.316	GG (9.10%)	GA (46.19%)	AA (44.71%)
rs12333117	6	5994992	Downstream	C/T	0.347	0.423	CC (32.92%)	CT (49.26%)	TT (17.82%)
rs582186	6	6001381	Intronic	A/G	0.450	0.371	GG (38.54%)	GA (48.87%)	AA (12.59%)
rs645649	6	6004959	Intronic	C/G	0.449	0.324	GG (45.25%)	GC (44.75%)	CC (10%)
rs582262	6	6007991	Upstream	C/G	0.480	0.230	GG (60.36%)	GC (33.76%)	CC (5.88%)
rs10484320	6	6010437	Upstream	C/T	0.152	0.219	CC (59.61%)	CT (36.15%)	TT (4.24%)
rs4960155	6	6010539	Upstream	C/T	0.425	0.493	CC (26.1%)	CT (46.04%)	TT (27.86%)
rs9405890	6	6012721	Intergenic	T/C	0.376	0.320	TT (47%)	TC (41.30%)	CC (11.7%)
rs1475157	6	6017169	Intergenic	A/G	0.164	0.181	AA (68.38%)	AG (27%)	GG (4.62%)
BDNF gene									
rs6265 (Val66Met)	11	27598369	Exonic	A/G	0.201	0.237	Val/Val (59.10%)	Val/Met (34.22%)	Met/Met (6.68)

[a]The less frequent allele (minor allele) is placed second.
[b]MAF refers to Minor Allele Frequency observed in the 1000 Genomes project (Abecasis et al. 2012).
[c]MAF observed in the current sample

· Is the variability of NRN1 gene associated with BSI psychopathological dimensions?

Among the six BSI psychopathological dimensions, the genetic variability of *NRN1* gene was related to depressive dimension and total BSI scores. In particular, the SNP rs1475157 was significantly associated with depressive symptoms (p=0.001556-genotypic model). Specifically, GG homozygotes showed higher scores on BSI depressive dimension than A allele carriers: 2.4 (1.08) and 1.8 (0.71), respectively (β=0.62 p=0.00036-recessive test). In addition, the total score on the BSI was also significantly higher in those individuals carrying two copies of the G allele: 10.84 (3.56) and 9.51 (2.68), respectively (β=2.51 p=0.00033 – recessive model). These associations remained significant after permutation analysis.

· Is the variability of NRN1 gene (SNP rs1475157) associated with cognitive performance?

The same genotype within rs1475157 polymorphism was also associated with cognitive performance. A linear trend was detected between the GG genotype and a worse cognitive performance in the following tests: a) Phonemic Fluency: GG 15.05(3.73) vs A carriers 16.72(4.11), β=-1.66 p=0.086; b) WCST total correct response: GG 65.52(5.45) vs A carriers 70.20(9.04), β=-4.5 p=0.029. However, these results did not remain significant when multiple testing corrections were applied.

· Is there a relationship between depressive dimension and cognitive performance?

We explored the relationship between those cognitive test observed to have a nominal association with *NRN1* and BSI depressive dimension and total scores. A trend correlation was found between BSI depression dimension scores and Phonemic fluency (β=-0.02 p=0.059). Also, higher BSI total scores were negatively correlated with lower phonemic fluency (β=-0.09 p=0.007). No relationship was found between WCST total correct response and BSI depressive dimension or total scores. When correcting for multiple testing, only the relationship between phonemic fluency and BSI total scores remained significant.

·Is the relationship between *NRN1* and depressive symptoms/cognitive performance modulated by the *BDNF*?

We finally tested the interaction between rs1475157 *NRN1* and the polymorphism Val66Met of *BDNF* gene on: i) depressive symptoms, ii) phonemic fluency and total correct responses WCST. First, a significant two-way interaction was found on the presence of depressive symptoms (β= 1.22 p=0.001) (Table 3, Figure 1A). In other words, carriers of both the GG genotype of rs1475157 *NRN1* and the *BDNF* Met allele presented significantly more depressive

symptoms. Second, we found a significant two-way interaction on phonemic fluency (β=-4.462 p=0.033) (Table 3, Figure 1B); meaning that carriers of both the GG genotype of rs1475157-*NRN1* and Met allele of the rs6265-*BDNF* presented significantly worse phonemic fluency performance. Third, no interaction effect was detected between on WCST scores (correct responses). After multiple testing, only the interaction between *NRN1xBDNF* on depressive symptoms was significant.

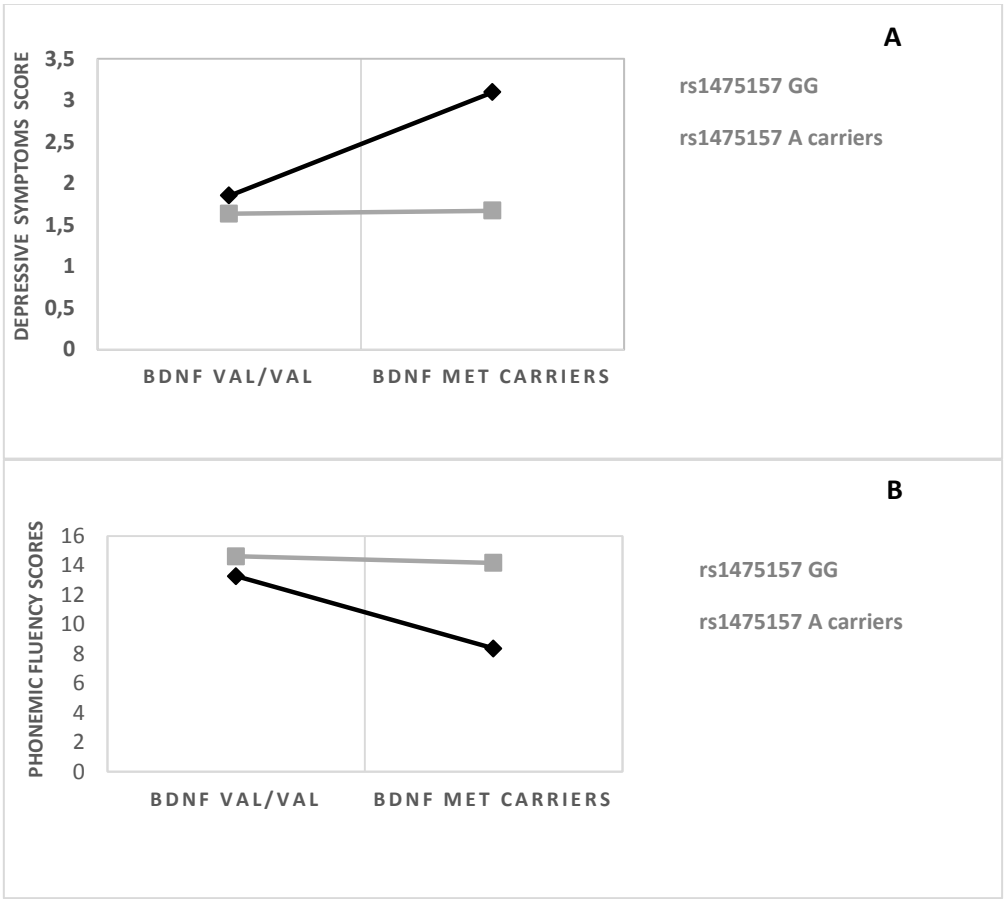

Figure 1. A) Graphical representation of the interaction effect between rs1475157 of *NRN1 (GG vs A carriers)* and rs6265 of *BDNF* (Val/Val vs Met carriers) on the presence of depressive symptoms, corrected by age and sex.

BSI scores $\widetilde{X}$ (sd): GG + Val/Val: 2.02(0.9); GG + Met carriers: 3.25(1.11); Met carriers + A carriers: 1.82(0.69); Val/val + A carriers: 1.79(0.75). **B)** Graphical representation of the interaction effect between rs1475157 of *NRN1* and rs6265 of *BDNF* on Phonemic Fluency scores, corrected by years of education and sex.

Phonemic Fluency $\widetilde{X}$ (sd): GG + Val/Val: 13.27(0.9); GG + Met carriers: 8.36(1.11); Met carriers + A carriers: 14.17(0.69); Val/Val + A carriers: 14.62(0.75).

Table 3. Linear regression models testing the main effects and interaction of *NRN1* (rs1475157) and BDNF (rs6265) on the presence of depressive symptoms **(A)** and phonemic fluency **(B).**

Outcome: **A) BSI Depressive Dimension**			
	β	**SE**	***P-value***
i) Main effects:			
NRN1 (rs1475157)(GG vs A carriers)	0.62	0.18	0.001
BDNF (rs6265) (Val/Val vs Met carriers)	0.09	0.80	0.246
Sex	0.09	0.07	0.248
Age	-0.008	0.01	0.439
ii) Interaction: *NRN1* BDNF*	1.22	0.38	**0.001**
B) Phonemic Fluency			
	β	**SE**	***P-value***
i) Main effects:			
NRN1 (rs1475157)(GG vs A carriers)	-1.349	0.992	0.175
BDNF (rs6265) (Val/Val vs Met carriers)	-0.451	0.426	0.290
Sex	-0.211	0.423	0.618
Education years	0.119	0.058	0.042
ii) Interaction: *NRN1* BDNF*	-4.462	2.089	**0.033**

A) i) Adj-R^2 =0.03 ii) Adj-R^2 =0.06
B) i) Adj-R^2 =0.009 ii) Adj-R^2 =0.018
β, regression coefficient; SE, standard error

Discussion:

Our study aimed to explore the role of Neuritin-1 gene on the expression of psychopathological dimensions and on the performance variability on executive function tasks in healthy subjects from the general population. Moreover, we were also interested in investigating whether the role of *NRN1* on these clinical and cognitive phenotypes is modulated by the *BDNF* gene (Figure 2).

First, our results suggest that *NRN1* gene variability is associated with depressive sub-clinical symptomatology. To our knowledge, this is the first work describing a genetic association of *NRN1* gene with depressive symptoms in a general population sample. Specifically, we have identified that the polymorphism upstream of rs1475157-*NRN1* shows a significant effect on the appearance of depressive symptoms, with individuals carrying the genotype GG showing higher scores compared to A allele carriers. These results are in line with the findings from animal models in which *NRN1* is shown as an interesting new player in depression. In this regard, knockdown of *NRN1* mice models in the hippocampus produced depressive-like behaviours (Son et al. 2012), whereas electroconvulsive therapy and antidepressant treatment produced changes in Neuritin levels (Alme et al. 2007; Dyrvig et al. 2014). In all, these results suggest the role of NRN1 in modulating depressive symptomatology and highlights this gene as a potentially interesting new target for antidepressant treatment.

Secondly, the same genotype within rs1475157 polymorphism showed a trend with a worse performance in phonemic fluency and WCST total correct responses. Although, these results did not remain significant after multiple testing correction, they are suggestive of an involvement of *NRN1* in executive function in the general population. The two previous studies on *NRN1* and cognition have detected such effect in SZ patients but not in healthy subjects (Chandler et al. 2010; Fatjó-Vilas et al. 2016); however, they analysed general intelligence and not executive function. In particular, Chandler et al found that the G allele of rs1475157 was associated with poorer performance in the abstraction component and IQ decline specifically in SZ patients; while the opposite allele of rs1475157 (A) located within an haplotype was associated with better IQ scores and later age at onset for SZ (Fatjó-Vilas et al. 2016).

Although, our findings are done in the context of a healthy sample, it is attractive to speculate about the interest of studying genetic variability in non-clinical populations in which there is a continuum distribution of the pathophysiological dimensions (Verdoux and Van Os 2002). The fact that *NRN1* has a role in the presence of depressive symptoms and with the neurocognitive performance can also be supported considering that it is widely accepted that depression is

associated with a number of neurocognitive deficits (Austin et al. 2001)(Christensen et al. 1997). In our study, we have also observed that there is a negatively relationship between BSI depression dimension scores and Phonemic Fluency performance. This is in line with the evidences reporting that patients with depression produce fewer words on fluency tasks (Fossati et al. 2003). From an intermediate phenotypes framework, it is of mention that these cognitive deficits have also been found in healthy first degree relatives of patients with either MDD or SZ (Christensen et al. 2006; Barrantes-Vidal N, 2007). Thus, our findings could support the importance of studying executive functions performance as a vulnerability marker for depression in the general population.

Despite the connection between the NRN1 rs1475157 and the risk for mental disorders is still unclear, the consideration of the putative effects of the analysed polymorphic sites on gene expression regulatory mechanisms represents a valuable resource to provide additional meaning and importance to our association data. Although rs1475157 is not a functional SNP, recent data has revealed the importance of intronic and intergenic variants as regulatory elements of gene expression (Dunham et al. 2012). The impact of non-coding variants of the *NRN1* SNPs can be examined using HaploReg (Ward and Kellis 2012), which is a tool that uses LD information from the 1000 Genomes Project to provide data on the predicted chromatin state of the queried SNPs, their sequence conservation mammals and their effect on regulatory motifs. Interestingly, rs1475157 is predicted to alter various regulatory motifs such as *HP1-site-factor* which is a telomere-capping protein whose function is necessary for the chromosome stability (Fanti et al. 1998) and it is involved in gene silencing (Jones et al. 2000). Moreover, there is also evidence that this SNP is a binding site for the circadian rhythm-related transcription suppressor E4BP4 (Mitsui et al 2001). This transcription factor is involved in the circadian expression of Per2, which is one of the essential components of mammalian circadian clocks (Ohno et al. 2007). Interestingly, depressive disorders have been related with a deregulation of the circadian biological clock that controls the neuronal physiological processes (Landgraf et al. 2014), which gives indirect support to our findings.

Third, our data shows for the first time that the effect of *NRN1*-rs1475157 in either the appearance of depressive symptoms or cognitive function performance is not independent of *BDNF* polymorphism. In other words, rs1475157-*NRN1* GG genotype that are carriers of the *rs6265-BDNF* Met allele present more depressive symptoms than the individuals carrying other combinations. Moreover, the same genotype combination (GG genotype of *NRN1*-rs1475157

and Met allele of *rs6265-BDNF)* was associated with poorer cognitive performance in terms of phonemic fluency scores. Then, to understand this synergistic effect, beyond the above-described effects of *NRN1*, the BDNF role has to be considered. Met-allele carriers have a significantly lower activity-dependent expression of *BDNF* (Egan et al. 2003) and it has been also related with a plausible increased risk for developing depression (Buchmann et al. 2013). In addition, the Met allele has been also linked with impaired episodic memory, working memory, and reduced hippocampal volume and function in healthy populations (Egan et al., 2003; Dempster et al., 2005; Tan et al., 2005)(Frodl et al. 2007), which all supports our findings.

Despite the fact that evidence of a statistical interaction does not necessarily map directly onto biological interaction, this finding is based on a previously described effects of *BDNF* on *NRN1* regulation (Naeve et al. 1997). This interaction is also supported if we considered evidences about that there is a positive correlation expression between both genes (BrainCloud: http://braincloud.jhmi.edu/) (Colantuoni et al. 2011). Moreover, these gene-gene interaction results are in line with another study reporting the interplay between *NRN1* and *BDNF* on the risk for developing Schizophrenia Spectrum Disorders (SSD) (Fatjó-Vilas et al 2016).

Since both neurotrophins are critically essential for a correct brain function plasticity, although the molecular mechanisms underlying this interaction is unknown, we could modestly hypothesize that both genes contribute synergistically to the modification of the correct synaptic plasticity which could have an impact in the underlying mechanisms of either the presence of depressive symptoms or cognitive performance alterations.

Our study should be interpreted in the context of some limitations. First, the moderate sample size to detect genetic associations should be mentioned, replication in larger samples from general population with higher statistical power are needed to confirm these findings. Second, the characteristics of the sample need to be considered when generalizing the present findings. Although the sample is drawn from the general population, representativeness is also limited by these characteristics. Third, when multiple testing is considered, only the association between *NRN1* and BSI scores and the interaction between *NRN1xBDNF* on depressive symptoms remain significant. However, the Bonferroni correction is often considered to be overly strict and conservative (Feise 2002; Gelman et al. 2012). Fourth, the evaluation of the psychopathological outcome could have benefited by using some interview-based complementary instruments. Fifth, other current factors not controlled in the present study, social adjustment or quality of life may influence the mood state of participants at the

time of assessment for depressive symptoms dimension. Sixth, it should be mentioned that as the genotype combination includes the minor alleles of both *NRN1*-rs1475157 and *rs6265-BDNF,* the interpretation of our results is hampered by the frequency of this combination in the population. Seventh, the variation of R^2 from the non-interaction models to the interaction was small but significant; thus this effect seems not of dismissible interest, since is it known that the power to detect interactions is typically lower than the power to detect main effects (McClelland and Judd 1993). In summary, the interpretation of these results should be done with caution and further studies are required to determine the biological mechanisms underlying not only the role of *NRN1* but also the detected gene interaction effect between *NRN1* and *BDNF* in a non-clinical sample.

To conclude, our results contribute to the understanding of the genetic heterogeneity present in the general population, suggesting that *NRN1* has a role in the appearance of depressive symptoms and cognitive performance in a non-clinical sample. Moreover, its effects seem to be modulated by *BDNF* gene, supporting a gene-gene interaction between both neurotrophic factors. Although new studies are needed to better understand the role of *NRN1* gene, our findings add support to the pleiotropic effect of *NRN1*, a neurotrophic factor with multiple roles in neurodevelopment and synaptic plasticity.

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CONTRIBUTORS

CP, LF and MFV designed the study. BA, GO, IR, JM designed and coordinated the evaluation protocol and conducted the sample recruitment. CP and MFV undertook the genetic/statistical analyses and wrote the first draft of the manuscript. All authors advised on interpretation of the results and contributed, read and approved the final manuscript.

Disclosure Statement

All authors report no biomedical financial interests or potential conflicts of interest

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Figures

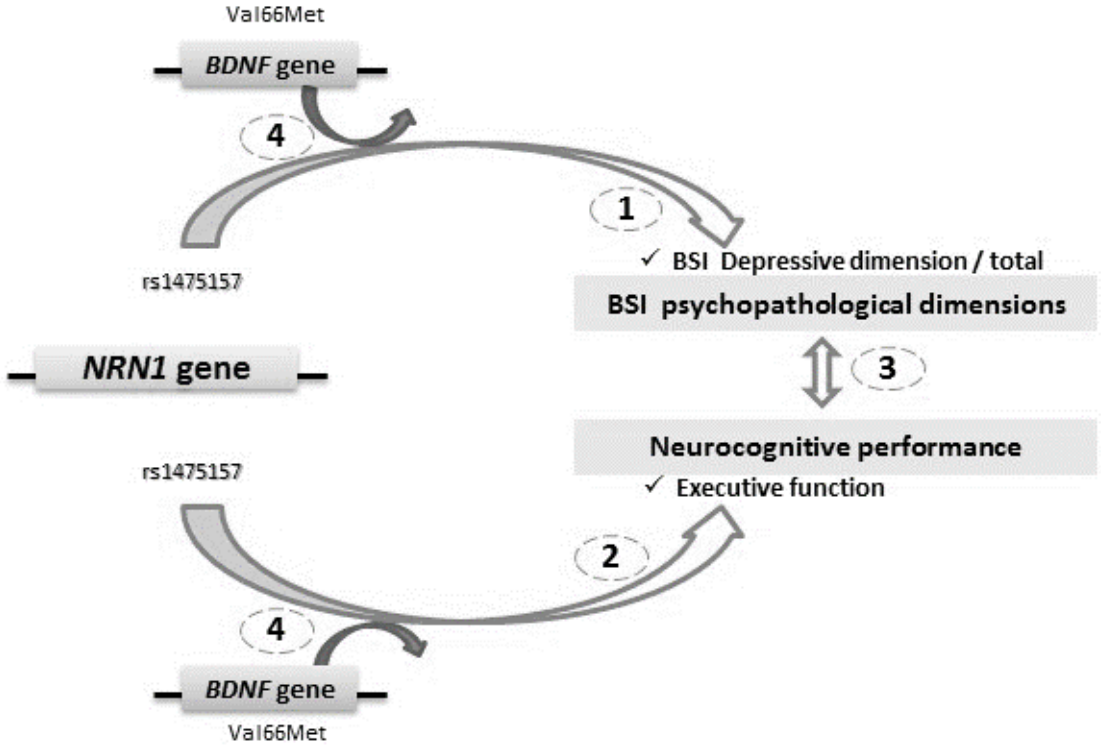

Figure 2. Graphical representation of the different steps analyses and findings of the study. 1) To explored whether the variability of *NRN1* gene is a*ssociated with BSI psychopathological dimensions, **2)*** To explored whether the variability of *NRN1* gene is *associated with of executive function performance, **3)*** *To explore if there was a relationship between depressive dimension and cognitive performance, **4)*** To analyzed whether the association between either *NRN1*-psychopathological profile or *NRN1*-cognitive performance is moderated by *BDNF* gene.