TITLE: Involvement of NRN1 gene in schizophrenia-spectrum and bipolar disorders and its impact on age at onset and cognitive functioning.

RUNNING TITLE: NRN1 in schizophrenia-spectrum and bipolar disorders

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ABSTRACT

Objectives. Neuritin 1 gene (*NRN1*) is involved in neurodevelopment processes and synaptic plasticity and its expression is regulated by Brain-Derived Neurotrophic Factor (BDNF). We aimed to investigate the association of *NRN1* with schizophrenia-spectrum disorders (SSD) and bipolar disorders (BPD), to explore its role in age at onset and cognitive functioning, and to test the epistasis between *NRN1* and *BDNF*.

Methods. The study was developed in a sample of 954 SSD-BPD patients and 668 healthy subjects. Genotyping analyses included eleven SNPs in *NRN1* and one functional SNP in *BDNF*.

Results. The frequency of the haplotype C-C (rs645649-rs582262) was significantly increased in patients compared to controls (p=0.0043), while the haplotype T-C-C-T-C-A (rs3763180-rs10484320-rs4960155-rs9379002-rs9405890-rs1475157) was more frequent in controls ($p=3.1x10^{-5}$). The variability at *NRN1* was nominally related to changes in age at onset and to differences in intelligence quotient, in SSD patients. Epistasis between *NRN1* and *BDNF* was significantly associated with the risk for SSD/BPD (p=0.005).

Conclusions. Results suggest that: i) *NRN1* variability is a shared risk factor for both SSD and BPD, ii) *NRN1* may have a selective impact of on age at onset and intelligence in SSD, and iii) the role of *NRN1* seem to be not independent of *BDNF*.

KEYWORDS: schizophrenia-spectrum and bipolar disorders, *NRN1*, age at onset, intelligence, *BDNF*.

INTRODUCTION

Schizophrenia and bipolar disorder are psychiatric disorders characterized by a prevalence of ~2-3%, which increases up to 3.5% when other affective and non-affective psychotic disorders such as schizoaffective or schizophreniform disorders are also included (Perala et al. 2007). A growing body of research suggests that schizophrenia-spectrum disorders (SSD) and bipolar disorder (BPD) share several epidemiological, clinical, neurobiological and genetic characteristics, raising important questions about the boundaries and distinctiveness of these psychiatric disorders.

On the one hand, they have a number of symptoms in common particularly in acute episodes, with regard to the presence of psychotic symptoms; their age at onset is quite similar; and, although there must be neurochemical differences, several findings emphasise the likelihood of dopamine dysregulation in both (Murray et al. 2004). Available evidence also supports that a generalized deficit is present across SSD and BPD, even though quantitative differences may exist (Hill et al. 2013). In view of these similarities and taking into account that schizophrenic and affective symptoms do not have a taxonic structure, the integration of categorical and dimensional approaches has been suggested of particular interest to the complete understanding of psychotic disorders (Peralta and Cuesta 2007).

On the other hand, an important genetic overlap between SSD and BPD has been classically reported by both epidemiological (Gottesman 1991; Lichtenstein et al. 2006) and molecular studies (Owen et al. 2007). More recently, the genome-wide approaches have evidenced a substantial shared polygenic contribution involving thousands of common genetic variants of small effect to the aetiology of these disorders (Lee et al. 2013).

These shared genetic risk factors, along with the clinical and cognitive similarities, have led to the notion that these severe mental disorders can be placed in the same etiopathological continuum, probably representing different phenotypic manifestations of common underlying processes.

In the search for specific genetic factors related to these disorders, studies face a number of challenges that arise from genetic and phenotypic complexity of these disorders. To this respect, it has been recently indicated that combining disorders with similar genetic risk profiles improves power to detect shared risk loci (Ruderfer et al. 2014). Similarly, the genotype-phenotype based approaches and the use of features with strong etiological significance have been suggested as a useful strategy to reduce heterogeneity and to identify specific genetic factors associated with such traits (Rasetti and Weinberger 2011; Swerdlow et al. 2015).Then the observed variability on traits such as cognitive impairments and age at onset among patients may reflect differences in the distribution of etiological factors and possibly also differences underlying vulnerability. To this respect, heritability estimates indicate that genetic factors contribute significantly to age at onset of psychotic symptoms (Hare et al. 2010) and to general cognitive functioning (Deary et al. 2009). Moreover, cognitive impairments are present in 70% of the patients with schizophrenia (Palmer et al. 1997) and twin studies have shown that a large genetic overlap underlies the observed comorbidity between these two phenotypes (Toulopoulou et al. 2007; 2010). Also, the earlier forms of these disorders usually present severe clinical and cognitive expression, high incidence of treatment refraction and poor outcome (Rapoport et al. 2005; Joseph et al. 2008). Accordingly, the

cognitive and clinical traits associated to age at onset may provide leads for recognizing and studying biological differences across diagnostic boundaries (Ongur et al. 2009).

Linkage data have provided positional evidence implicating the short arm of chromosome 6 in the risk for SSD and also in their associated cognitive deficits (Straub et al. 1995; Schwab et al. 1995; Hallmayer et al. 2005). The most studied gene included in this chromosomal region is Dysbindin-1 gene (*DTNBP1,* 6p22.3), which has been consistently associated with SSD and BPD (Schwab and Wildenauer 2009) as well as with age at onset and cognitive deficits (Wessman et al. 2009; Fatjo-Vilas et al. 2011). Also in this region, and far less explored, there is the Neuritin 1 gene (*NRN1*, 6p25.1) also called candidate plasticity-related gene 15 (cpg15) (Nedivi et al. 1993). During early embryonic development, *NRN1* is expressed in multiple brain regions and acts as a survival factor for neural progenitors and differentiated neurons (Putz et al. 2005). Later in development, *NRN1* promotes growth and stabilization of axonal and dendritic arbors along with synapse formation and maturation (Cantallops et al. 2000; Javaherian and Cline 2005). *NRN1* continues to be expressed in the adult brain, where its expression is correlated with activity-dependent functional synaptic plasticity (Corriveau et al. 1999; Harwell et al. 2005; Flavell and Greenberg 2008). Furthermore, the expression of *NRN1* is regulated by neurotrophins such as Brain-Derived Neurotrophic Factor (*BDNF*, 11p13) (Naeve et al. 1997; Karamoysoyli et al. 2008). BDNF promotes the differentiation and growth of developing neurons in central and peripheral nervous systems (Buckley et al. 2007) and its intracellular distribution and activity-dependent secretion is altered by the Met variant of a functional polymorphism in the *BDNF* gene, which consists of a valine (Val) substitution for methionine (Met) at codon 66

(Val66Met). Interestingly, *BDNF* gene polymorphisms have been associated with clinical symptoms –such as age at onset- and cognitive functioning in both SSD and BPD (Krebs et al. 2000; Rybakowski et al. 2006).

According to all the above mentioned, *NRN1* was already considered a candidate gene for neurodevelopment disorders by Chandler et al. (2010), who reported the effect of *NRN1* polymorphic variation on general intelligence impairments in patients with schizophrenia. We considered the interest of investigating the implication of *NRN1* in the aetiology not only of schizophrenia, but also across the schizophrenia-spectrum and bipolar disorder continuum. Moreover, we also aimed to extend the previous study on the relationship of *NRN1* with cognitive impairments by testing the effect of this gene on age at onset, a characteristic that is related to cognitive performance.

As synaptic plasticity alterations have been suggested to be present both in SSD and BPD (Craddock et al. 2006), we hypothesized that sequence variability of the gene would be related to the risk for developing any of these disorders. Considering the described involvement of *NRN1* in cognitive processes, we also hypothesized that *NRN1* gene could exert its effect not only by modulating general cognitive functioning, but also age at onset. Finally, given that *NRN1* is a BDNF-regulated gene, we explored the statistical epistasis between *NRN1* and *BDNF* as a proxy analysis of their involvement in common biological pathways.

MATERIALS AND METHODS

Sample

The patients' sample comprised 954 individuals of Spanish Caucasian origin. They were drawn from consecutive admissions to three Child and Adolescent Psychiatry Units and four Adult Psychiatric Units, and were evaluated by experienced psychiatrists. All of them met the DSM-IV-TR diagnosis criteria: 73% schizophrenia-spectrum disorders (49% schizophrenia, 11% schizophreniform disorder, 8% schizoaffective disorder, 5% Psychotic Disorder NOS) and 27% bipolar disorder I or II. Exclusion criteria included: age above 65 years, major medical illnesses that could affect brain functions, substance-induced psychotic disorder, neurological conditions, history of head trauma with loss of consciousness and having at least one parent not from Spanish Caucasian origin. Patients were diagnosed based on the following schedules: KSADS (Kaufman et al. 1997) for patients up to 17 years of age, and SCID (First et al. 1997) or CASH (Andreasen et al. 1992) for adult patients. Age at onset of the first episode was determined by means of these clinical schedules and/or the SOS inventory (Perkins et al. 2000).

The control sample consisted of 668 Spanish Caucasian unrelated adult healthy individuals. They met the same exclusion criteria as patients. They were recruited from university students and staff, and their acquaintances, plus independent sources in the community. They were interviewed and excluded if they reported a history of mental illness and/or treatment with psychotropic medication.

All participants provided written consent after being informed about the study procedures and implications. In the case of patients below the age of 18, written 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 was approved by the local ethics committee of each participating centre. All procedures were carried out according to the Declaration of Helsinki.

Neurocognitive assessment

The general cognitive performance was evaluated in 607 patients and in 476 healthy subjects. Intellectual quotient (IQ) was estimated using the Block Design and Vocabulary or Information subtests of the WAIS-III (Wechsler 1997) or WISC-IV (Wechsler 2004), in accordance with the method suggested by Sattler (2001). Cognitive assessment was carried out by experienced neuropsychologists. In patients, the cognitive evaluation was conducted when stabilization of symptoms and readiness for cognitive evaluation was decided by the clinical team.

Molecular Analyses

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

Coverage of *NRN1* genomic sequence and ~10kb upstream and downstream was achieved by including 11 tag SNPs (Table 1). 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 study were also considered. The SNP rs6265 (Val66Met) at *BDNF* gene was also genotyped. 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% and all were in Hardy-Weinberg equilibrium.

TABLE 1

Statistical analyses

All data were processed using SPSS 21.0 software (SPSS IBM, New York, U.S.A). The program Haploview v4.1 (Barrett et al. 2005) was used to estimate the Hardy-Weinberg equilibrium and the linkage disequilibrium (LD) between *NRN1* SNPs (Figure S1 in Supplementary Material). By means of using the Solid Spine criteria three haplotype blocks were identified (Block 1: SNP1-SNP3, Block 2: SNP4-SNP5 and Block 3: SNP6-SNP11) and a sliding window analysis was conducted within each block.

The genetic power was calculated using Epi-info-v3.5.1 (Dean et al. 1991) by assuming an additive model, a disease prevalence of 3% and minor allele frequencies in accordance with those observed in sample. All markers had an 80% power to detect a genetic effect with an OR≥1.2.

Case-control associations were analysed using the Unphased-v3.1.4 (Dudbridge 2003), using a cut-off threshold for rare haplotypes of 1%. A 10000 permutations procedure was applied to all tests to limit type II error. The odds ratios (OR) were estimated from the absolute number of alleles/haplotypes estimated in patients and controls (EpiInfo-v3.5.1).

Additive models as implemented in Plink 1.07 (Purcell et al. 2007) were used to conduct lineal regression analyses to explore the relationship between *NRN1* and age at onset and IQ. First, the relationship between the *NRN1* and age at onset was tested in the complete patients' sample (including gender and diagnosis group as covariates) and

also separately in each group (adjusted by gender). Second, the relationship between the *NRN1* and IQ was tested in the complete patient's sample (including age at onset, months of evolution and diagnosis group as covariates) and also separately in SSD, BPD (adjusted for age at onset and months of evolution) and controls. PLINK's max(T) permutation procedure with 10000 iterations was performed.

The effect of *NRN1* and *BDNF* interaction was tested both on: i) the risk for developing SSD or BPD, ii) age at onset (adjusted for sex and diagnosis) and IQ (adjusted for age at onset and months of evolution), in patients. Epistasis was explored using the model based multifactor dimensionality reduction (MB-MDR) approach by applying 'mbmdr' R-package (Calle et al. 2010). This method merges multi-locus genotypes in order to overcome the dimensionality problem and to increase the power to detect gene interactions associated with disease or phenotype. It also allows adjusting for confounding effects and correcting for multiple testing by 1000 permutations approach. In all analyses, the significance cut-off was established at p-value of 0.05.

RESULTS

Sample characteristics

Table 2 shows the main sociodemographic and clinical data of the sample. Variables that showed differences between groups were used as covariates when appropriate (see statistical analyses section).

TABLE 2

Association analysis of *NRN1* and schizophrenia-spectrum and bipolar disorders

There were no differences between sampling groups as regards the genotypic distribution of each polymorphism (data not shown), and genotype frequencies showed no gender differences within groups (patients and controls; data not shown).

SNP1 (G allele), SNP4 (C allele) and SNP5 (C allele) were significantly more frequent among patients compared to controls (χ^2=4.81 p=0.028, χ^2=5.05 p=0.024 and χ^2=8.04 p=0.004, respectively). After multiple correction adjustment only the association of SNP5 remained significant (OR(95%CI)=1.27(1.07-1.49), empirical p-value=0.044)).

Haplotypes associated with SSD and BPD are given in Table 3. The frequency of the haplotype G-C (Block 1: SNP1-SNP2) and haplotype C-C (Block 2: SNP4-SNP5) was significantly increased in patients than in controls. The result in Block 2 remained significant after permutation procedure; then, this haplotype was considered a risk haplotype for SSD and BPD. On the contrary, the haplotype T-C-C-T-C-A (Block 3: SNP6-SNP11) had higher frequencies in controls. Results in Block 3 also remained significant after multiple testing and could be considered as reflecting a protective effect of this haplotype. Note that other haplotypes included in the haplotype in Block 3 were

also detected (Table S1 in Supplementary Material). These results remained essentially unchanged when only SSD patients and controls were included.

TABLE 3

***NRN1* and age at onset of the disorders**

Patients carrying two copies of the T allele at SNP2 (15.33%) presented a lower age at onset than those not carrying this allele (β=-0.772 p=0.029). Patients homozygous for the C allele of SNP10 (7.80%) also showed later age at onset than those not carrying this allele (β=0.918 p=0.016). The haplotype C-A (SNP10-11) was associated with age at onset: (β=0.956 p=0.015) and also several haplotypes within Block 3 (all including the C-A haplotype) (Table S2 in Supplementary Material).

When the same analysis was conducted only including SSD patients, the results for SNP10 and haplotype SNP10-11 remained significant while SNP2 did not (Table S3 in Supplementary Material). In an additive way, carrying two copies of the haplotype C-A was associated with later SSD age at onset (Figure 1A). However, these results were not significant after permutation procedure. No association was detected within BPD patients' group.

***NRN1* and cognitive functioning**

In SSD patients, the same haplotypes within Block 3 contributed to IQ scores (Table S4 in Supplementary Material). A linear trend was detected between the number of copies of these haplotypes and higher IQ scores (Figure 1B), meaning that subjects carrying these haplotypes showed better general cognitive performance than non-carrier subjects. However, after permutation analyses these results did not remain significant. No

significant association with IQ was detected between these polymorphisms either in the whole patients' sample, in BPD or in healthy subjects.

FIGURE 1

Epistasis between *NRN1 and BDNF*

Two order gene-gene interaction models were developed and revealed the effect of the *BDNF* Val/Val genotype in combination with different *NRN1* variants (SNP1 (GG: β=0.654 p=0.001), SNP3 (AA: β=0.514 p=0.003) and SNP9 (TG: β=0.457 p=0.0004)) on an increased risk for developing both SSD and BPD. In contrast, *BDNF* Met/Met was associated with a lower risk in combination with *NRN1* SNP2 (TT: β=-2.185 p=0.0052). After permutation analysis, the interaction $BDNFxNRN1_{SNP9}$ remained significant (p=0.005). No significant epistatic effect was detected on age at onset and IQ after permutation.

DISCUSSION

This case-control based approach adds to the only one previous Neuritin 1 gene association study developed by Chandler et al. (2010) in a sample of 336 patients with schizophrenia and 172 controls. Unlike Chandler and collaborators, in our sample of 954 patients and 668 healthy subjects we report that *NRN1* sequence variability accounts for a modest proportion of the risk for these disorders. On the one hand, we have identified a two SNP haplotype (SNP4-SNP5: C-C) that is associated with the risk for these disorders. As expected, due to the polygenic architecture of the studied disorders, the effect of this haplotype is small although significant (OR=1.28). On the other hand, we have observed haplotypes in the 5' upstream region that have a protective effect. Although significance for these associations persisted after permutation procedure, the low frequency of the protective haplotypes in the population has to be considered when evaluating the attributable risk associated to these genetic variants.

The present study also provides new evidence of interest as regards understanding the heterogeneity in age at onset and cognitive performance of SSD and BPD. Our results suggest that *NRN1* variability has a role in SSD age at onset, pointing towards a specific effect on modifying neurodevelopment processes related to the time of emergence of these disorders. Although these results should be interpreted cautiously because they are only significant at an uncorrected level, it is interesting that the C allele of SNP10, which is included in the above described protective haplotype, is associated with a later age at onset of SDD. Then, taking into account that the 51% of SSD patients are carriers of this allele (358 C carriers vs 339 TT), together with the particularly poor prognosis

associated to schizophrenia in childhood and adolescence in contrast to the adult manifestation (Clemmensen et al. 2012), this modulatory effect is of non-dismissible potential clinical interest.

Our study also shows the association between this gene and intelligence in SSD. This selective impact of *NRN1* on intelligence in SSD may suggest its involvement in processes underlying cognitive functioning, which are described to be more quantitatively impaired in SSD (Hill et al. 2013). Again, although results did not reach significance after permutation, it is of potential interest that the haplotypes identified in the present study contain the same haplotype that Chandler et al. (2010) described to be associated with better fluid intelligence in schizophrenia patients and not in healthy subjects (SNP10-SNP11: C-A).

In all, our results suggest in a convergent manner that allelic variants in Block 3 of *NRN1* could represent a protective factor, not only due to their association to a reduction of the risk for SSD and BPD, but also because within patients, these variants are related to a later of age at onset and a better cognitive performance. This lends support to the notion that specific genetic variability could play a role in defining illness subgroups and points towards the interest of understanding the pathways from genotype to clinical phenotype, which will be crucial for new classification systems and to develop novel therapeutic strategies.

In further interpreting these results, it is necessary to consider the results obtained by whole genome approaches. To our knowledge, *NRN1* has not appeared as a significant locus in the published GWAS for schizophrenia and other related disorders. However, these negative results could be influenced, for example, by the small effect attributable

to common variants or by heterogeneity of the samples. It should also be considered that *NRN1* could be exerting its effect by means of modifying a more specific trait associated with psychotic disorders. In this regard, a genome-wide scan for intelligence conducted in a general population sample revealed suggestive linkage for IQ on 6p25.3-21.31 and already highlighted *NRN1* as a positional candidate gene (Posthuma et al. 2005). Moreover, a subtype of schizophrenia characterized by pervasive cognitive deficit was also linked to 6p25-p22 region (Hallmayer et al. 2005). More recently, a GWAS has established that common variants (SNPs) may account for 40-50% of intelligence variance (Davies et al. 2011) and in a GWAS-based pathway analysis has reported that general fluid intelligence appears to be characterized by genes affecting quantity and quality of neurons and therefore neuronal efficiency (Christoforou et al. 2014). Among the genes included in the top pathways identified in this study, there was the *BDNF*, a regulator of *NRN1* expression. According to all these data and given the described gradual increase in heritability of IQ from childhood to late adolescence (Deary et al. 2009; Bouchard 2013) and the reported early occurrence of intellectual impairment even years before the onset of the psychotic symptoms (Cannon et al. 2002), it is plausible that those genes that influence brain development, as *NRN1*, may be modulating illness traits, as IQ and age at onset, and ultimately influencing the risk for these disorders.

Although the connection between the *NRN1* sequence variability and the risk for SSD and BPD 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. 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 considered 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. As an example, SNP2 (rs12333117), associated with age at onset in the present study, is located in a downstream region, in a DNAse region (T-47D) and it is predicted to alter several motifs that overlap the recognition sequences of transcription factors such as AP-1/Jun, suggesting possible factor-factor interactions. There is also evidence that this SNP could modify the promoter histone mark H1, which plays an active role in the formation of epigenetic silencing marks (Yang et al. 2013). Another example refers to the SNP4 (rs645649), included in the identified risk haplotype and that is located in an intronic region where two proteins bound: SUZ12 (involved in methylation processes leading to transcriptional repression of the affected target genes) and ZNF263 (implicated in basic cellular processes as a transcriptional repressor). Furthermore, several resources provide information about the correlation between genotype and tissue-specific gene expression levels, which may help in the interpretation of molecular genetics association studies (GTEx Project, www.gtexportal.org (Lonsdale et al. 2013); BrainCloud, http://braincloud.jhmi.edu/ (Colantuoni et al. 2011)). In this regard, variations in NRN1 expression have been associated with SNPs along the gene. Therefore, although functional studies are needed, the association of *NRN1* sequence variants with SSD and BPD phenotypes could be linked to the final availability or functionality of the protein which, in turn, could

dysregulate *NRN1* role on neurite outgrowth and arborization and/or on neuronal processes associated with plasticity.

Finally, based on the analyses of epistasis between *NRN1* and *BDNF*, our data suggest that the interaction between the Val/Val genotype (*BDNF*) and the TG genotype (*NRN1*, *SNP9:* rs9379002) could modulate the risk for SSD and BPD. Despite the fact that evidence of a statistical interaction as we report here does not necessarily map directly onto biological interaction, it is of note that it is based on a previously described effects of BDNF on NRN1 regulation (Naeve et al. 1997). Then, it could be hypothesised that the reported functional effects of the *BDNF* Val66Met polymorphism could impact on NRN1 availability or function, explaining therefore the gene-gene interaction on the risk for developing SSD and BPD and contributing to understand the controversial results associated to single gene analyses. To this respect, some studies have implicated the *BDNF* Val allele in these disorders and, as the Val allele is associated with increased synaptic plasticity and growth (Egan et al. 2003), it has been suggested that this allele could promote increased synaptic connections between certain brain regions that underpin common symptoms. However, recent meta-analyses have failed to confirm the direct association of Val66Met polymorphism with the risk for schizophrenia (Zhao et al. 2015) or bipolar disorder (Gonzalez-Castro et al. 2014). On the other hand, taking into account that BDNF exerts a direct impact on neuronal growth and plasticity in the limbic system (Conner et al. 1997; Rattiner et al. 2004), it is of note that G allele carriers of rs9379002 (SNP9, *NRN1*) show higher *NRN1* expression than TT homozygotes in the hypothalamus (GTEx Project). Then, we could speculate that higher expression of both *BDNF* and *NRN1* could be underlying the detected epistatic risk

effect. To this respect, it is remarkable that a case-report study suggested the relationship between a duplication of *NRN1* gene (i.e. increased gene dosage) and the white matter and neurocognitive abnormalities observed in one patient (Linhares et al. 2015). Accordingly, we would have expected to detect the association not only with the heterozygous TG genotype but also with the GG. This lack of significant interaction could be explained by the low frequency of GG genotype (7%) and the corresponding low frequency of the combination of Val/Val x GG (*BDNFxNRN1*$_{SNP9}$*)*. Therefore, although further studies are needed, these results are in line with recent trends in the field of molecular genetics, which consider the importance of testing gene networks rather than isolated gene effects for better understanding the gene-phenotype relationship in complex disorders (Gilman et al. 2012). Nonetheless, the fact that the SNP9 is included in the protective haplotype while it is detected to exert a risk effect when interacts with Val/Val genotype could suggest that the effect of this SNP may differ depending on the genetic background in which the alleles are present (Moore 2003). Moreover, beyond gene-gene interactions, the effect of environmental factors should also be considered. In this regard, the fact that *NRN1* is classified as an immediate early gene (IEG) (Loebrich and Nedivi 2009), meaning that it can be rapidly induced by extracellular stimuli and act as a transcription factor on downstream targets, highlights the interest of analysing the combined effect of *NRN1* and *BDNF* in gene-environment studies.

Some limitations of this study must be acknowledged. First, the controls' age range is partially overlapped with the age range of incidence of SSD and BPD. However, due to the fact that personal psychiatric history and treatment was discarded, the percentage of

false negatives would be very low and should not interfere with the obtained results. Second, the polygenic nature of mental disorders and the minor effect of the common genetic variants limit the power of our sample size, especially in the case of the analyses split by diagnosis. In line with this, although the use of features with strong etiological significance has been suggested as a useful strategy to increase the power to detect genetic effects, the power of the analyses targeting age at onset and neurocognition is reduced due to the non-availability of data in all subjects. This statistical power reduction could be related with the loss of significant effects after permutation procedures. Third, the antipsychotic treatment was not specified and, therefore, cognitive analyses, although covaried by age at onset and months of evolution, were not adjusted by treatment type or duration. Fourth, in spite of the interest of the selected polymorphism at *BDNF* due to its functional effects, future studies should include other genetic variants along this gene. Lastly, although the permutation procedures have been applied, if multiple testing is addressed for the overall analyses not all the findings would remain significant. Then, although results cannot be dismissed completely, since they come from a directed hypothesis and they are partially in line with a previous study (Chandler et al. 2010), their interpretation should be conducted with caution and replication studies are needed.

Overall, our results contribute, from a biological approach, to the understanding of the genetic mechanisms involved in SSD and BPD and also of the relationship between genetic variability and the clinical heterogeneity of these disorders. Then, our findings suggest the role of Neuritin 1 gene as a mixed susceptibility/modifier gene (Fanous and Kendler 2008), which increases the susceptibility to these disorders and modifies certain

presentations. However, new studies should be developed to further acknowledge the involvement of *NRN1* and its interaction with other genes in the aetiology of these mental disorders.

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Table 1. SNPs genotyped in Neuritin 1 gene (*NRN1,* chromosome 6p25.1, from 598233 to 6007633 bp). The table includes the dbSNP number, the genomic and gene position and the alleles of the 11 SNPs genotyped along the gene (UCSC Genome Browser on Human Mar. 2006 Assembly (hg18), http://genome.ucsc.edu/cgi-bin/hgTracks). [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).

	SNP	Chr	Position	Region	Distance from SNP1	Distance from previous SNP	Alleles[a]	MAF[b]
SNP1	rs2208870	6	5992490	Intergenic			A/G	0.333
SNP2	rs12333117	6	5994992	Downstream	2502	2502	C/T	0.402
SNP3	rs582186	6	6001381	Intronic	8891	6389	A/G	0.393
SNP4	rs645649	6	6004959	Intronic	12469	3578	C/G	0.356
SNP5	rs582262	6	6007991	Upstream	15501	3032	G/C	0.273
SNP6	rs3763180	6	6009848	Upstream	17358	1857	G/T	0.437
SNP7	rs10484320	6	6010437	Upstream	17947	589	C/T	0.236
SNP8	rs4960155	6	6010539	Upstream	18049	102	T/C	0.492
SNP9	rs9379002	6	6012391	Intergenic	19901	1852	T/G	0.27
SNP10	rs9405890	6	6012721	Intergenic	20231	330	T/C	0.309
SNP11	rs1475157	6	6017169	Intergenic	24679	4448	A/G	0.176

Table 2. Sample description and statistical comparisons between patients and controls. Proportion (%) or mean scores (standard deviation) are given. (SSD: schizophrenia-spectrum disorders, BPD: bipolar disorders)

	All Patients (n=954)	**SSD** (n=697)	**BPD** (n=257)	**Controls** (n=668)
Male (%)	65.6 %	71.2 %	50.6 %[+]	46.7 %[*]
Age at interview	32.33 (13.10)	31.79 (12.83) [+]	33.9 (13.71)[+]	27.05 (9.99)[*]
Years of education	10.13 (4.06)	9.58 (3.82)	11.98 (4.29)[+]	13.87(2.87)[*]
Age at onset	21.54 (6.47)[a,b]	20.72 (5.33)[a]	23.88(8.53)[a,+]	-
Months of evolution	146.24 (137.6)	140.35 (140.07)	162.93 (129.25)	-
Current IQ	89.80 (15.26)[c]	89.02 (15.37)[c]	92.86 (14.48)[c, +]	99.48 (13.64)[c*]

(a) Information about age at onset was available for the 73.5% of patients (74.3% SSD and 71.2% BPD).
(b) 35.29% were classified as early-onset (first psychotic episode occurred before 18 years of age).
(c) Information about IQ was available for 63.6% of patients (69.4% SSD and 47.8% BPD) and 71.25% of healthy subjects.
(*) Controls differed significantly from patients ($p<0.001$)
(+) BPD patients differed significantly from SSD patients ($p<0.03$)

Table 3. *NRN1* most significant haplotypes associated to the risk for schizophrenia-spectrum and bipolar disorders. Frequency estimates in patients and controls, significance levels and OR of the case-control comparison are given.

SNP1	rs2208870	G		
SNP2	rs12333117	I		
SNP3	rs582186	C		
SNP4	rs645649		C	
SNP5	rs582262		I	
SNP6	rs3763180		C	T
SNP7	rs1048432			I
SNP8	rs4960155			C
SNP9	rs9379002			I
SNP10	rs9405890			C
SNP11	rs1475157			I
				A
Ca- Freq [a]		34.3	25.9	0.1
Co- Freq [b]		30.7	21.4	1.5
χ^2		4.26	7.99	17.45
OR (CI 95%) [c]		1.18 (1.01-1.37)	1.28 (1.08-1.51)	0.09 (0.02-0.37)
Global p- value		0.11	0.038	0.001
Individual haplotype p - value		0.037[‡]	0.0043*	0.000031**

[a] Ca - Freq refers to each haplotype frequency within cases

[b] Co - Freq refers to each haplotype frequency within controls

[c] Chi-squared tests and Odds ratio (OR) were estimated from the absolute number of observed haplotypes in cases and controls

[‡] No significant after performing 10000 permutations, *adjusted p value from permutation test p* = 0.1748

* Significant adjusted level based on 10000 permutations, *adjusted p value from permutation test p* = 0.0219

** Significant adjusted level based on 10000 permutations, *adjusted p value from permutation test p* = 0.002

Figure 1. Relationship between *NRN1* and age at onset and IQ in SSD patients. Linear regression graphs showing the relationship between SSD patients' *NRN1* haplotypes and: (A) age at onset, (B) IQ. For illustration purposes, the haplotype dump option was used to estimate individual haplotype phases. Considering only those haplotypes estimated with a probability ≥95%, each subject was defined according to its haplotype dose. A) The most significant *NRN1* haplotype associated to age at onset (SNP10-11: C-A, β=0.89 p=0.019) was selected and SSD patients were classified as: 47.01% non-carriers (0), 45.41% one-copy carriers (1) and 7.58% two-copy carriers (2). B) The haplotype C-T-G-C-A (SNP7-11) was selected to represent graphically the detected association between *NRN1* variability and IQ within SSD patients (β=4.02 p=0.022). Patients were classified as: 82.2% non-carriers (0), 16.9% one-copy carriers (1) and 0.9% two-copy carriers (2).