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Original article

Evidence of an epistatic effect between Dysbindin-1 and Neuritin-1 genes on the risk for schizophrenia spectrum disorders

C. Prats [a,b], B. Arias [a,b], J. Moya-Higueras [b,c], E. Pomarol-Clotet [b,d], M. Parellada [b,e,f,g], A. González-Pinto [b,h], V. Peralta [i,j], M.I. Ibáñez [b,k], M. Martín [l], L. Fañanás [a,b], M. Fatjó-Vilas [a,b,d,*]

[a] Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals. Facultat de Biologia, Universitat de Barcelona, Institut de Biomedicina de la Universitat de Barcelona (IBUB), Barcelona, Spain
[b] Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Madrid, Spain
[c] Department of Psychology, Faculty of Education, Psychology and Social Work, University of Lleida, Spain
[d] FIDMAG Germanes Hospitalàries Research Foundation, Barcelona, Spain
[e] Servicio de Psiquiatría del Niño y del Adolescente, Hospital General Universitario Gregorio Marañón, Madrid, Spain
[f] Instituto de Investigación Sanitaria del Hospital Gregorio Marañón (IiSGM), Madrid, Spain
[g] Departamento de Psiquiatría, Facultad de Medicina, Universidad Complutense, Madrid, Spain
[h] BIOARABA Health Research Institute, OSI Araba, University Hospital, Psychiatry Service, University of the Basque Country (EHU/UPV), Vitoria, Spain
[i] Servicio de Psiquiatría, Complejo Hospitalario de Navarra, Pamplona, Spain
[j] Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain
[k] Department of Basic and Clinical Psychology and Psychobiology, Universitat Jaume I, Castelló, Spain
[l] Adolescent Unit, CASM Benito Menni, Sant Boi de Llobregat, Spain

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ABSTRACT

Background: The interest in studying gene–gene interactions is increasing for psychiatric diseases such as schizophrenia-spectrum disorders (SSD), where multiple genes are involved. Dysbindin-1 (*DTNBP1*) and Neuritin-1 (*NRN1*) genes have been previously associated with SSD and both are involved in synaptic plasticity. We aimed to study whether these genes show an epistatic effect on the risk for SSD.
Methods: The sample comprised 388 SSD patients and 397 healthy subjects. Interaction was tested between: (i) three *DTNBP1* SNPs (rs2619537, rs2743864, rs1047631) related to changes in gene expression; and (ii) an haplotype in *NRN1* previously associated with the risk for SSD (rs645649-rs582262: HAP-risk C-C).
Results: An interaction between *DTNBP1* rs2743864 and *NRN1* HAP-risk was detected by using the model based multifactor dimensionality reduction (MB-MDR) approach ($P = 0.0049$, after permutation procedure), meaning that the risk for SSD is significantly higher in those subjects carrying both the A allele of rs2743864 and the HAP-risk C-C. This interaction was confirmed by using a logistic regression model ($P = 0.033$, OR (95%CI) = 2.699 (1.08–6.71), $R^2 = 0.162$).
Discussion: Our results suggest that *DTNBP1* and *NRN1* genes show a joint effect on the risk for SSD. Although the precise mechanism underlying this effect is unclear, the fact that these genes have been involved in synaptic maturation, connectivity and glutamate signalling suggests that our findings could be of value as a link to the schizophrenia aetiology.

1. Introduction

Complex genetic disorders with a polygenic architecture such as schizophrenia involve multiple genes of small effect, which interact with different social and environmental factors. Several genes combine with other genes influencing the control of a trait variation. In this sense, the interest in studying gene–gene interactions is increasing because of the enhanced power to elucidate the biological processes underlying mental disorders [1].

* Corresponding author at: Secció Zoologia i Antropologia Biològica, Facultat de Biologia, Universitat de Barcelona, Av Diagonal 643, 08028 Barcelona, Spain; FIDMAG Germanes Hospitalàries Research Foundation, Av Jordà 8, 08035 Barcelona, Spain. Tel.: +34 936 529 999x1490.
E-mail addresses: mar.fatjovilas@ub.edu, mfatjo-vilas@fidmag.com (M. Fatjó-Vilas).

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In regard to these biological processes, a growing body of evidence has established that synaptic plasticity is an inherent feature of brain function during both development and adulthood. Recent studies highlight the convergence of several genes associated with schizophrenia-spectrum disorders (SSD) onto a coherent biological pathway at the synapse, with a specific role in plasticity [2]. Therefore, focusing on genes and their interactions implicated in synaptic plasticity may provide better understanding of SSD pathogenesis.

Dysbindin-1 gene (*DTNBP1*) is described as a modulator of the synaptic transmission homeostasis [3] due to its pre- and post-synaptic roles. Dysbindin-1 interacts pre-sinaptically with proteins involved in vesicular trafficking and exocytosis regulating the neurotransmitters release [4]. Additionally, Dysbindin-1 regulates the formation and function of the postsynaptic density (PSD), a set of proteins that interacts with the postsynaptic membrane to provide structural and functional regulatory elements for neurotransmission and for NMDA receptors [5]. Schizophrenia patients show lower levels of Dysbindin-1 mRNA in dorsolateral prefrontal cortex and hippocampus compared to healthy subjects [6,7]. This reduction in Dysbindin-1 expression has been related to a decreased glutamate output [5] and to an increased dopamine release [8]. Moreover, it has been reported that *DTNBP1* expression levels are modulated by common DNA sequence changes (Single Nucleotide Polymorphisms, SNPs) [9,10]. Several studies based on SNP analyses have shown an association between *DTNBP1* and SSD (i.e. [11–13]).

Interestingly, the chromosome region 6p25-p22, in which *DTNBP* is located, has been recurrently linked to schizophrenia and intelligence [14–16] and it also includes the Neuritin-1 gene (*NRN1*, also called candidate plasticity gene 15). *NRN1* is an immediate early gene that is induced by synaptic activity through activation of NMDA receptors [17] and it is regulated by brain-derived neurotrophic factor (*BDNF*) and neurotrophin-3 [18]. As reviewed by Zhou and Zhou [19], *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. Basic activity-related changes in the central nervous system are thought to depend on neuritin-mediated modification of synaptic transmission, especially in the hippocampus and neocortex [20]. Two previous studies have analyzed common variants along *NRN1* sequence and have shown its association with intelligence and SSD [21,22], suggesting that certain allelic variants at this gene modulate the risk for developing SSD and the general cognitive performance.

Based on the above mentioned, both *DTNBP1* and *NRN1* are: (i) implicated in plasticity processes, (ii) expressed in hippocampus and cortical neurons, and (iii) playing a role in the glutamatergic signalling through NMDA receptors, whose hypofunction has been associated with the neurobiology of schizophrenia [23]. Taking into account the common pathway in which both genes are involved, we aimed to study whether these genes work in concert and collectively contribute to increase the risk of SSD.

2. Methods

2.1. Sample

The sample comprised 785 Caucasian subjects: 388 SSD patients (mean age (sd) = 26.94 (11.38), 74.7% males) and 397 healthy unrelated subjects (mean age (sd) = 24.38 (6.54), 44.6% males). Patients' diagnoses (DSM-IV-TR evaluated with KSADS, SCID and/or CASH) were as follows: 66.5% schizophrenia, 16.7% first episode psychotic or psychotic disorder not otherwise specified, 11.9% schizophreniform disorder and 4.9% schizoaffective disorder. This sample is partially overlapped with the ones included in previous studies [12,22].

Exclusion criteria were: major medical illnesses that could affect brain functions, substance-induced psychotic disorder, neurological conditions, and history of head trauma with loss of consciousness. All participants provided written consent after being informed about the study procedures and implications.

2.2. SNP selection and genotyping

Genotyping of all SNPs was performed using Taqman 5′ exonuclease assays. First, three *DTNBP1* SNPs were selected due to their previous association with changes in the expression of *DTNBP1* in brain [7]: SNP1: rs2619537 (5′ flanking region), SNP2: rs2743864 (intronic), SNP3: rs1047631 (3′UTR). The fact that SNPs related to changes in availability or function of the protein can have stronger penetrance on the phenotype adds relevance in these SNPs selection criteria for studying their interaction. *DTNBP1* genotypes were dichotomized according to the described allelic association linked to expression changes: rs2619537 (C carriers vs AA), rs2743864 (A carriers vs GG), rs1047631 (G carriers vs TT).

Second, two *NRN1* SNPs (rs645649 (intronic) – rs582262 (upstream)) were selected. Despite these SNPs have not been associated with changes in the gene expression, they were considered of interest due to the previous observation of the increased frequency of the corresponding risk haplotype C-C in patients with SSD compared to healthy subjects [22]. Then, analyses were conducted with the risk haplotype derived from these two SNPs, classifying participants in two groups: (i) HAP-risk C-C carriers, which included individuals with at least one risk haplotype, and (ii) HAP-risk C-C non-carriers. Although *NRN1* SNPs are located in non-coding sequences, recent data has revealed the importance of intronic and intergenic variants as regulatory elements of gene expression [24]. More specifically, these two SNPs have been described as having putative influence on biological processes by changing the binding affinity of different transcription factors and binding proteins.

2.3. Statistical analysis

Comparisons of gender between patients and controls were performed by the chi-squared test. Hardy–Weinberg equilibrium and the linkage disequilibrium (LD) were estimated using Haploview v4.1. As linkage disequilibrium (LD) between *DTNBP1* SNPs showed low pairwise D'/r^2, all analyses were conducted by using *DTNBP1* SNPs separately. The two SNPs at *NRN1* showed a strong LD ($D' = 0.85$). Then, individual *NRN1* haplotypes were estimated using Unphased-v3.1.4 and, considering only those assigned with a probability $\geq$95%, each subject was classified according to the haplotype dose (HAP-risk C-C carriers vs non-carriers).

First, an epistasis screening was conducted by using the model based multifactor dimensionality reduction (MB-MDR) by applying 'mbmdr' R-package [25]. This method merges multi-locus genotypes into a one dimension construct that reflects the risk and can be represented by 3 values: high, low and non-informative. Thus, this method overcomes the dimensionality problem and increases the power to detect gene interactions associated with disease or phenotype. It also allows applying a 10,000 permutation procedure to the analyses besides adjusting for gender.

Second, the detected interaction by MB-MDR was confirmed by using a logistic regression model (SPSS 21 software, SPSS IBM, New York, U.S.A.), similarly to other studies that have explored joint effects of two genes (i.e. [26–29]). This complementary methodology allows analysing the magnitude of the interaction effect and