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Replication of previous genome-wide association studies of psychiatric diseases in a large schizophrenia case–control sample from Spain

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ABSTRACT

Genome wide association studies (GWAS) has allowed the discovery of some interesting risk variants for schizophrenia (SCZ). However, this high-throughput approach presents some limitations, being the most important the necessity of highly restrictive statistical corrections as well as the loss of statistical power inherent to the use of a Single Nucleotide Polymorphism (SNP) analysis approach. These problems can be partially solved through the use of a polygenic approach. We performed a genotyping study in SCZ using 86 previously associated SNPs identified by GWAS of SCZ, bipolar disorder (BPD) and autistic spectrum disorder (ASD) patients. The sample consisted of 3063 independent cases with DSM-IV-TR diagnosis of SCZ and 2847 independent controls of European origin from Spain. A polygenic score analysis was also used to test the overall effect on the SCZ status. One SNP, rs12290811, located in the *ODZ4* gene reached statistical significance ($p = 1.7 \times 10^{-4}$, Allelic odds ratio = 1.21), a value very near to those reported in previous GWAS of BPD patients. In addition, 4 SNPs were close to the significant threshold: rs3850333, in the NRXN1 gene; rs6932590, at MHC; rs2314398, located in an intergenic region on chromosome 2; and rs1006737, in the CACNA1C gene. We also found that 74% of the studied SNPs showed the same

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tendency (risk or protection alleles) previously reported in the original GWAS ($p < 0.001$). Our data strengthen the polygenic component of susceptibility to SCZ. Our findings show *ODZ4* as a risk gene for SCZ, emphasizing the existence of common vulnerability in psychosis.

1. Introduction

The common disease/common variant (CDCV) hypothesis posits that genetic vulnerability to common complex disorders is mainly due to common genetic variants, which have a modest effect on the disease risk and are shared by different subpopulations. The additive effect of these low-risk variants, together with environmental factors and their interactions, will therefore cause the disease (Risch and Merikangas, 1996; Reich and Lander, 2001). Thus, the search for these common risk variants has led to the development of genome-wide association studies (GWAS), hypothesis-free association studies that allow the testing of several thousand single nucleotide polymorphisms (SNPs) simultaneously (Wellcome Trust Case Control Consortium, 2007). However, the success of finding risk variants for common disorders can only be understood through the use of several large, representative samples of patients and controls.

The development of GWAS has been particularly fruitful in the field of psychiatric genetics, as they have spurred the development of new hypotheses and the identification of new pathways potentially involved in these diseases. Specifically, GWAS have identified common genetic variations related to schizophrenia (SCZ) (Stefansson et al., 2009), bipolar disorder (BPD) (Sklar et al., 2008), and autism spectrum disorder (ASD) (Weiss et al., 2009), which meet modern standards for replication and significance. Genome-wide genetic approaches generate a large amount of data that can be used for comparisons and to search for risk variants for different neuropsychiatric conditions with shared etiological factors, such as SCZ, BPD, and ASD (O'Donovan et al, 2009; Sullivan et al., 2012). This has been the case for SCZ and BPD (International Schizophrenia Consortium et al., 2009), where some common risk factors for these disorders have been found using GWAS (Ferreira et al., 2008; O'Donovan et al., 2008; Williams et al., 2011; Steinberg et al., 2014). Regarding the relationship between ASD and other neuropsychiatric disorders, evidence of shared genetic risk factors has been published in copy number variation studies, with *NRXN1* among the most interesting candidates (O'Donovan et al., 2009).

Based on the results of GWAS for neuropsychiatric disorders, a number of candidate genes have been suggested, but additional replication in several independent samples is required to verify the original findings. Nevertheless, GWAS present some disadvantages and difficulties. The most common analysis in GWAS is to perform independent associations for each SNP. However, this approach has several flaws. One of them is that the need for corrections due to multiple rounds of testing for thousands of SNPs sets a very low and restrictive significance threshold. Furthermore, putative low-risk variants are likely to interact with each other to influence the phenotype; therefore, an independent analysis of each SNP most likely causes a loss of power to detect their effects. The genetic etiology of some common diseases could be explained by exploring a number of variants simultaneously (Valdar et al., 2006). For psychiatric disorders, this can be carried out with a polygenic approach (International Schizophrenia Consortium et al., 2009).

The objective of this work is to analyze in a large and homogenous (all of European origin from Spain) sample of patients with schizophrenia, the most significant polymorphisms that have been associated with psychosis (SCZ, BPD) and autism in previous GWAS. The advantage of this approach is that a relatively small number of tests are needed in comparison with traditional GWAS.

2. Methods

2.1. Samples

The study consisted of 2847 DNA control samples (57% males) and 3063 DNA samples from patients with the diagnosis of SCZ (56% males). The statistical power to detect positive results, calculated with Quanto software (Gauderman and Morrison, 2006), was between 42% and 98% (Supplementary Fig. 1). All the samples were taken from the Spanish National DNA Bank of the Spanish National Network for Research in Mental Health CIBERSAM. The samples came from 11 different research groups (Supplementary Table 1). The mean age was 43.61 ± 15.54 (range: 17–91) for the control samples and 39.04 ± 29.50 (range: 14–87) for the case samples. All patients met the DSM-IV-TR criteria for SCZ diagnosis. The assessment methods in patients and controls are indicated in Table S1. All the individuals involved in this study gave their written consent for this study. The study was approved by the ethics committee of each group's institution.

2.2. SNP selection and genotyping

A total of 95 SNPs were selected from 19 previous GWAS published between 2007 and 2009 on SCZ, BPD, and ASD. GWAS were selected using the Schizophrenia Gene Database and PubMed (Supplementary Table 2).

The SNP selection criteria were (i) minor allele frequency >0.1 and (ii) the most significant SNPs in each study, including not only the genome-wide significant SNPs but also the significant SNPs close to the threshold. These 95 SNPs were used as the input for Spectro DESIGNER software (Sequenom, San Diego, CA, USA) to generate multiplex assays for genotyping. Twenty SNPs were discarded by the above-mentioned tool or presented a low likelihood of successful design according to the software, and these were replaced by linked SNPs with $r^2 > 0.8$ in the HapMap samples from Utah (USA) residents with northern and western European ancestry (CEU). By using this strategy, a total of 86 SNPs were included in three high-level multiplexes (Supplementary Table 2) and were genotyped using the iPLEX MassARRAY technology from Sequenom. The results were manually inspected to confirm genotype assignments. Exclusion criteria for the SNPs were the following: (i) a genotyping call rate lower than 95%, (ii) a significant difference in call rates between cases and control subjects ($p < 0.05$), and (iii) departure from Hardy–Weinberg equilibrium (HWE) in control samples ($p < 0.05$). As a genotyping quality control, two trios from the Coriell Institute included within the HapMap CEU samples were genotyped. There were no Mendelian inconsistencies, and all genotypes were consistent with HapMap data.

2.3. Control for population stratification

In the Spanish population, no stratification has been detected, except in the Canary Islands (Gayán et al., 2010; Laayouni et al., 2010). No individuals from the Canary Islands were included in this study (Table S1). Therefore, genetic association studies in our sample have a minor risk of population stratification. Nevertheless, as an additional control, a subset of 5365 samples was genotyped for 47 ancestry-informative markers using the Sequenom MassARRAY. Structure 2.3.1 software (Pritchard et al., 2000; http://pritch.bsd.uchicago.edu/structure.html) was used under the admixture model with 50,000 replications for the burn-in period for parameter estimations to estimate the percentage of European ancestry using the HapMap CEU and Yoruba in Ibadan,

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