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Brief report

Case-control association study of 36 single-nucleotide polymorphisms within 10 candidate genes for major depression and bipolar disorder

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ABSTRACT

In this study we investigated 36 single nucleotide polymorphisms within 10 genes previously associated with major depression and bipolar disorder, as well as with the response to their treatment (ABCB1, ABCB4, TAP2, CLOCK, CPLX1, CPLX2, SYN2, NRG1, 5HTR1A and GPRIN2). No association with mood disorders and clinical outcomes was observed.

1. Introduction

The heritability of major depression (MD) is estimated to be as high as 30-40% (Kendler et al., 2006) and that of bipolar disorder (BD) is estimated to be as high as 60-85% (Smoller and Finn, 2003). Despite their strong genetic aetiology, little is known about the specific genes underlying these disorders (Kato, 2007; Burmeister et al., 2008). Therefore, this report, we focused on 40 single-nucleotide polymorphisms (SNPs) within 10 genes that could potentially be involved with the etiology of MD and BD as well as with response to their treatment. The examined genes were *CLOCK* (circadian loco-motor output cycles kaput protein), *ABCB1* (ATP-binding cassette, sub-family-B [MDR/TAP], member-1), *ABCB4* (ATP-binding cassette, sub-family-B [MDR/TAP], member-4), *TAP2* (transporter-2, ATP-binding cassette, sub-family-B [MDR/TAP]), *CPLX1* (complexin1), *CPLX2* (complexin2), *SYN2* (synapsinII), *NRG1* (neuregulin1), *5HTR1A* (5-hydroxytryptamine (serotonin) receptor-1A), and *GPRIN2* (G-protein regulated inducer of neurite outgrowth-2).

Polymorphisms within *CLOCK* have repeatedly been found to be associated with susceptibility to both MD and BD (Mendlewicz, 2009; Lamont et al., 2010). Also, *ABCB1* variants are related to the severity of MD and with clinical remission in patients treated with some serotonergic drugs (Lin et al., 2011). While there are no published association studies linking variants within *ABCB4* with psychiatric disorders, *ABCB4* is adjacent to *ABCB1*, and significant linkage disequilibrium exists between the two genes (Leschziner et al., 2006). *TAP2* also appears to be associated with major psychiatric disorders (Fellerhoff and Wank, 2009). Additionally, *CPLX1* and *CPLX2* expression is altered in patients with MD and BD (Eastwood and Harrison, 2000; Sawada et al., 2002; Knable et al., 2004), and altered expression of *SYN2* has repeatedly been observed in patients with BD and schizophrenia (Vawter et al., 2002; Saviouk et al., 2007). Variants of *NRG1* have recently been associated with BD (Green et al., 2005; Thomson et al., 2007; Prata et al., 2009), schizophrenia, and MD (Bertram et al., 2007). Some genetic studies have also shown a possible association of *5HTR1A* with MD and BD (Vincent et al., 1999; Savitz et al., 2009; Kishi et al., 2011). Finally, *GPRIN2* encodes for a protein that binds to activated G (0) alpha-protein, a membrane-bound protein that may have a role in the growth of neuritis (Chen

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et al., 1999). Of note, according to current evidence, not only single SNPs but also specific haplotypes have been linked with the susceptibility to mood disorders and with the response to their treatment (Mendlewicz, 2009; Popova and Kulikov, 2010).

Based on the current evidence, the aim of this study was to investigate possible associations of several SNPs and related haplotypes within *ABCB1*, *ABCB4*, *TAP2*, *CLOCK*, *CPLX1*, *CPLX2*, *SYN2*, *NRG1*, *5HTR1A* and *GPRIN2* with MD and BD, and clinical response to treatment in a sample of Korean in-patients treated with antidepressants or mood stabilizers.

2. Methods

A complete description of the whole sample has been reported elsewhere (Serretti et al., 2011). Briefly, the study sample included 145 patients with MD and 132 with BD (diagnosed according to DSM-IV criteria (Sheehan et al., 1998)) who were consecutively recruited at the Department of Psychiatry at the Catholic University of Korea College of Medicine, Seoul, Korea. We also included a sample of 170 psychiatrically healthy Korean control subjects who underwent the same assessment of MD and BD subjects.

All patients admitted to the hospital were assessed for illness severity at baseline and at discharge by means of the Hamilton Rating Scale for Depression (HAMD) (Hamilton, 1960) for patients with MD and the Young Mania Rating Scale (YMRS) (Young et al., 1978) for patients with BD.

The main outcome measures were: 1) differences in genotype and allelic frequencies among MD, BD and healthy control subjects, and 2) influences of the forty 40 SNPs under investigation on clinical improvement in the two groups of psychiatric patients separately analyzed. Further outcomes included the effects of the selected SNPs on treatment response and remission rates. Based on previous studies, treatment response was defined *a priori* as a $\geq 50\%$ reduction from baseline to discharge in the two rating scales (Hirschfeld et al., 2004; Riedel et al., 2010). Remission was defined as a HAMD score ≤ 7 at discharge for patients with MD (Riedel et al., 2010) and as a YMRS score ≤ 12 for patients with BD (Hirschfeld et al., 2004). The study protocol was approved by the Institutional Review Board (approval no. HC10TISI0031).

SNPs were mainly chosen among those that had been previously investigated in association with MD, BD or response to their pharmacological treatments, and among those that had a minor allele frequency (MAF) > 0.05 in Asian populations (data from http://hapmap.ncbi.nlm.nih.gov/) and that were relevant for pathways involved in the mood disorders. Genomic DNA was extracted from blood with standard methods and quantified. The high-throughput genotyping method using a pyrosequencer (Biotage AB, Sweden) was employed to genotype the 40 SNPs within the 10 genes under investigation (supplementary Table 1).

Statistical analyses were performed using the Statistica package (StatSoft, 1995). Categorical outcomes were calculated using the $\chi 2$ statistic. The influence of the SNPs under investigation on continuous outcomes was examined using analysis of variance (ANOVA).

Haploview-3.2 was used to generate a linkage-disequilibrium (LD) map and to test for Hardy-Weinberg equilibrium (HWE) (Barrett et al., 2005). Tests for associations using multi-marker haplotypes were performed using the statistics environment "R" (http://www.R-project.org) and package "haplo.score," to compare clinical and socio-demographic outcomes among different haplotypes. Permutations ($n=10{,}000$) were performed. All p-values were 2-tailed, and statistical significance was conservatively set at the 0.001 level (approximately corresponding to the Bonferroni correction for the 19 blocks of SNPs and the two diagnoses under investigation) in order to reduce the likelihood of false positive results.

With these parameters we had a sufficient power (0.80) to detect a small-medium effect size ($\omega=0.22$) for case-control analyses that corresponded, as an example, to an odds ratio of 1.53 between the two groups of patients and the group of controls, and to detect medium-large ($d=0.35$ and $d=0.37$) effect sizes. For patients with MD and BD, respectively, who carried the CC genotype of rs2243404 as compared with those carrying the CT genotype (Cohen, 1988). This corresponded to the ability to detect final differences of 2.6 points in HAMD scores and of 2.3 points in YMRS scores.

3. Results

Socio-demographic features such as gender, age, and other clinical and socio-demographical variables are reported in supplementary Table 2. Since there were significant differences among the three groups of patients and controls with regard to age and gender (gender: $\chi^2=6.25$, $p=0.04$; age: $F=5.25$, $p=0.005$), age and gender were added as covariates in the following case-control analyses. Four SNPs were excluded because they were monomorphic (rs2229107) or because they were not in HWE (rs1801260, rs10042486 and rs307614). Strong LD was observed among several SNPs (data not shown, available on request). A separate analysis of MD and BD patients and of healthy subjects yielded similar results (data not shown, available on request).

There were no significant differences in allelic and genotype frequencies between MD and BD patients and healthy controls (supplementary Tables 3 and 4, all p-values > 0.001). Further, we did not observe any significant association between all the genetic variants and the HAMD and YMRS scores in patients with MD or BD, respectively (all p-values > 0.001). The haplotype analysis focusing on the sliding windows haplotypes also found no significant associations.

4. Discussion

We found no significant association between the investigated SNPs and related haplotypes, a diagnosis of MD or BD and clinical response to treatment in the present study. This suggests that the SNPs included in our study, considered both alone or in combination, are unlikely to affect disease phenotypes, clinical improvement, or other MD and BD clinical factors.

Our results are in contrast with several earlier studies focusing on single SNPs or specific haplotypes (Vincent et al., 1999; Cassidy et al., 2006; Saviouk et al., 2007; Mendlewicz, 2009; Prata et al., 2009; Wang et al., 2009; Lamont et al., 2010; Popova and Kulikov, 2010; Kishi et al., 2011; Lin et al., 2011). However, there may be several explanations for these discrepancies. Candidate gene studies, such as the present one, are associated with a high likelihood of false positive findings (Sullivan, 2007). In addition, our limited sample size raises concerns that the negative findings of this study could simply reflect the lack of statistical power to detect the small differences that are likely to be associated with single SNPs. A further limitation of our study is that we included subjects treated with drugs with different mechanisms of action. This does not allow us to draw definitive conclusions about the influence of the investigated SNPs on specific drugs or classes of drugs. Finally, incomplete coverage of the investigated genes should also be considered.

Based on our findings, future studies will need to include a higher number of SNPs covering larger portions of genes, larger samples and more homogeneous groups of drugs.

Conflict of interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.psychres.2012.11.009.

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