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Genes Involved in Neurodevelopment, Neuroplasticity and Major Depression: No Association for *CACNA1C*, *CHRNA7* and *MAPK1*

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Objective: Genetics factors are likely to play a role in the risk, clinical presentation and treatment outcome in major depressive disorder (MDD). In this study, we investigated the role of three candidate genes for MDD; calcium voltage-gated channel subunit alpha1 C (*CACNA1C*), cholinergic receptor nicotinic alpha 7 subunit (*CHRNA7*), and mitogen-activated protein kinase 1 (*MAPK1*).
Methods: Two-hundred forty-two MDD patients and 326 healthy controls of Korean ancestry served as samples for the analyses. Thirty-nine single nucleotide polymorphisms (SNPs) within *CACNA1C*, *CHRNA7*, and *MAPK1* genes were genotyped and subsequently tested for association with MDD (primary analysis) and other clinical features (symptoms' severity, age of onset, history of suicide attempt, treatment outcome) (secondary analyses). Single SNPs, haplotypes and epistatic analyses were performed.
Results: Single SNPs were not associated with disease risk and clinical features. However, a combination of alleles (haplotype) within *MAPK1* was found associated with MDD-status. Secondary analyses detected a possible involvement of *CACNA1C* haplotype in resistance to antidepressant treatment.
Conclusion: These data suggest a role for *MAPK1* and *CACNA1C* in MDD risk and treatment resistance, respectively. However, since many limitations characterize the analysis, the results must be considered with great caution and verified.

KEY WORDS: *MAPK1*; *CACNA1C*; *CHRNA7*; Major depressive disorder; Deep phenotyping.

INTRODUCTION

Major depressive disorder (MDD) is a severe psychopathological condition that has as main characteristic a severe alteration of mood and self-image, along with somatic and vegetative symptoms.[1)] The high disabling potential of this condition, as well as the growing number of cases worldwide, has led MDD to be one of the most investigated psychiatric disorders.[2)]

There is evidence of a genetic liability for MDD, and several genes have been associated with the disease in case-control and wide-genome studies. Despite this, little is known about the specific effects of the genes (and their alterations) on disease risk and other clinical features.[3,4)]

Among several investigated genes, calcium voltage-gated channel subunit alpha1 C (*CACNA1C*), cholinergic receptor nicotinic alpha 7 subunit (*CHRNA7*), and mitogen-activated protein kinase 1 (*MAPK1*) have raised interest, since they encode for products involved in key nodes of several brain processes related to MDD.[5-13)] To some extent, they are involved in dendritic development, neuronal survival, synaptic plasticity and memory/learning.[14-16)]

CACNA1C encodes for an L-typed voltage-dependent calcium channel subunit contributing to channels that influence neuronal excitability. Its potential role in MDD

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was already investigated in literature.[11-13] *MAPK1* encoded protein is implicated in a variety of biological processes and it has a critical role in synaptic and structural plasticity. *MAPK1* is also involved in the initiation and progression of inflammatory processes, which are highly related to depressive states.[5-7,17,18] *CHRNA7* encoded receptor is involved in cognition through interneuron modulation of dopamine and glutamate signaling. Evidence of its involvement in psychiatric disorders has also been reported, especially in antidepressant mechanisms.[8,9]

In a previous case-control study, we detected an association of *CACNA1C*, *MAPK1*, and *CHRNA7* genes with bipolar disorder (BPD) risk.[19] Further, in previous studies we detected a possible involvement on *CACNA1C* in antidepressant response[20] and an association of *MAPK1* with remission.[17] Given such evidence, here we further investigated these genes in MDD evaluating independent samples of patients and controls. In order to explore possible minor effects on specific disease characteristics, we also evaluated a potential impact on age of onset, history of suicide attempt, symptoms severity and treatment outcome.

METHODS

Subjects

Two-hundred and forty-two MDD patients according to the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) criteria[21] consecutively admitted to the Department of Psychiatry in Seoul St. Mary's Hospital for MDD treatment, and 326 healthy controls, were used as samples in this study. Recruitment details and exclusion criteria have been previously reported.[19] Briefly, patients were treated with paroxetine and/or venlafaxine and evaluated for antidepressant response approximately after six weeks of treatment. Controls were recruited among hospital staff and non-psychiatric hospital patients. In order to be considered controls they did not have to meet criteria for a major current psychiatric condition. Subjects with severe or unstable medical and/or neurological conditions, in treatment with a long-acting antipsychotic, with current or recent (past six months) comorbidity with alcohol and substance abuse disorders were not eligible for the study. All individuals were Koreans of Korean ascendants. The local ethical committee approved the study procedures and all the subjects were included after they had signed an informed consent.

Evaluations

The Mini-International Neuropsychiatric Interview (MINI)[21] was employed for psychiatric evaluations. Demographic and clinical variables, including age at first illness episode (onset) and history of suicide attempt, were collected by clinical interview and review of clinical charts. Lifetime suicide attempt was defined as a non-fatal self-injurious behavior with intent to die. Depressive severity was evaluated by the Hamilton Rating Scale for Depression (HAMD).[22] Response to treatment, remission and resistance to treatments were defined according to Schosser *et al.*[23] Briefly, response to treatment is defined by 50% improvement of HAMD scores from baseline to endpoint; remission by a HAMD score of 7 or less at the endpoint; resistance by non-response to at least two adequate consecutive antidepressant trials.[23]

Genetic Analysis

A total of 39 single nucleotide polymorphisms (SNPs) within *CACNA1C*, *CHRNA7*, and *MAPK1* genes were tested for association with MDD and clinical features (Supplementary Table 1). Analyses were performed by independent investigators who were blind to the status of the subjects. Samples showing ambiguous alleles on repeated genotyping were discarded. For details about gene selection and genotypization please refer to Calabrò *et al.*[19]

Statistical Analysis

Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested by Haploview 3.2 software for Windows.[24] Case-control analysis and genetic associations with clinical variables were analyzed using the IBM SPSS package for Windows ver. 23 (IBM Corp., Armonk, NY, USA). Haplotypes' analysis was performed in "R" environment (http://cran.r-project.org/), using the statistics package "haplo.score". SNPs were combined according to LD blocks. The sliding windows method was applied to narrow the block of SNPs analyzed for each LD Block. Further, we set the analyses to perform a minimum of 10,000 and a maximum of 50,000 simulations for each haplotype analyzed. The simulated p value is reported together with the global p value.

Finally, possible epistatic effects[25] were evaluated

through multifactor dimensionality reduction test (MDR, http://www.multifactordimensionalityreduction.org/). Further details on the methods used can be found in Calabrò *et al.*[19] To control for multiple testing, the false discovery rate method (FDR)[26] was employed to evaluate the significance of findings. Significance was considered for corrected $p < 0.05$.

RESULTS

Demographic Data

Sociodemographic data of the sample under investigation are reported in Supplementary Table 1. No significant differences between cases and controls were observed in terms of age and gender (respectively 38.0% and 54.9% females, aged 43.6 ± 15 years and 45.4 ± 13 years). In patients, mean age at onset was 39.8 (± 14) years. Fifty-four patients (22.3%) had a positive history of suicide attempt. In our samples, all the SNPs analyzed were in HWE (Supplementary Table 2). Supplementary Figure 1 reports the results of LD test.

Risk of MDD

In single SNP analyses, alleles and genotypes were not differentially distributed between cases and controls (all $p < 0.05$) (Supplementary Table 3). However, a block of alleles in *MAPK1* (rs8136867-rs9610417) was associated with MDD (global $p = 0.010$). In particular, the most frequent GC haplotype was associated with control-status, while the less frequent AT haplotype was associated with MDD-status (Table 1). Tests for epistatic effects (MDR) did not provide a significant model for MDD risk (data not shown, available on request).

Table 1. Association between rs8136867-rs9610417 *MAPK1* haplotypes with MDD*

rs8136867	rs9610417	Simulated p	p	Hap-score	Hap-freq
g	c	0.015	0.012	−2.512	0.592
a	c	0.329	0.337	0.960	0.304
a	t	0.013	0.013	2.482	0.102

MDD, major depressive disorder; Hap-score, haplotype score on phenotype; Hap-freq, haplotype frequency.
*Stat = 0.032 and global $p = 0.010$.

Symptoms Severity, Age of Onset and History of Suicide Attempt

Exploratory analyses did not provide evidence for any association between depressive severity, age of onset and suicide attempt history with the genetic variants considered in our study (Supplementary Table 4). By haplotypes analysis, trends of association with symptom severity at baseline (*CHRNA7* and *CACNA1C*) and discharge (*CACNA1C*) could be observed (Supplementary Table 5), but above the threshold of significance. Tests for epistasis (MDR) did not provide any significant model for these phenotypes.

Treatment Outcomes (Response, Remission and Resistance to Treatment)

Single SNPs were not significantly associated with measures of treatment outcome (all $p < 0.05$). However, a four-SNPs haplotype within *CACNA1C* (rs880342-rs11062196-rs2238062-rs3819536) was associated with treatment resistance (Table 2). Two SNPs (rs880342 and rs3819536) mainly contributed to treatment resistance, with the allelic CG combination being more frequent in the non-resistant group and the TA block being more common in the resistant group. However, the different distribution between the two groups was significant only when the central pair of SNPs (rs11062196 rs2238062) haplotype was GA. Tests for epistasis (MDR) did not provide a significant model for any of the investigated outcomes (response, remission and resistance).

Table 2. Association between 4 SNPs haplotypes in *CACNA1C* with treatment resistance in MDD patients*

rs880342	rs11062196	rs2238062	rs3819536	Simulated p	p	Hap-score	Hap-freq
c	g	a	g	0.019	0.019	−2.338	0.369
t	g	a	g	0.604	0.59	−0.539	0.079
c	g	a	a	0.722	0.717	0.362	0.288
t	g	a	a	0.001	0.001	3.348	0.095

MDD, major depressive disorder; Hap-score, haplotype score on phenotype; Hap-freq, haplotype frequency.
*Stat = 0.004 and global $p = 0.012$.

DISCUSSION

As a main finding, we found a block of alleles within *MAPK1* (rs8136867-rs9610417 haplotype) associated with MDD risk. In particular, the GC combination (more frequent in controls) might be protective, while the opposite AT combination (more frequent in MDD cases) might increase disease risk. The two SNPs (both intronic) are about 5 kb from each other, and do not seem to fall on methylation points. However, according to the Human Splicing Finder (http://www.umd.be/HSF3/), an online prediction software, mutation on these sites may alter enough the sequence to broke/insert specific motifs and thus alter, quantitatively or qualitatively, *MAPK1* product. The presence of both alterations may increase this effect (as supported by their analysis as a block). In particular, the AT variant might produce the loss of a potential splice site and 3 enhancer motifs. Of note, one of the SNPs included in the haplotype, rs8136867, was previously associated with treatment efficacy in Caucasian MDD subjects.[17] However, we could not replicate the association with treatment outcome in our sample.

As regards treatment outcomes, extending our previous results on an independent sample evaluated for different SNPs within *CACNA1C*,[20] in this study we identified a haplotype (rs880342-rs11062196-rs2238062-rs3819536) potentially associated with resistance to treatments, i.e. multiple non-response to adequate antidepressant treatment trials. Within this block, rs880342 and rs3819536 exerted a major influence on the treatment-resistant phenotype, with CG variant being protective while TA more frequent in the resistant patients group. These variations may cause some changes to the sequence, since they both alter a CG sequence with a possible impact on methylation mechanics. Further, an analysis with Human Splicing Finder for consensus motifs revealed that the TA haplotype loses 3 enhancer motifs and it produce a possible slice site and an additional silencer motif. As such, an effect of this haplotype on *CACNA1C* seems plausible. This data adds to our previous results linking *CACNA1C* alterations with antidepressant efficacy.[20]

We did not find any significant epistatic model in this sample, suggesting that these different genes work independently from each other in MDD risk. This result slightly differs from that we obtained in BPD patients,[19] where SNPs in these different genes seems to have synergic effects (rs1016388 within *CACNA1C*, rs1514250, rs2337980, rs6494223, rs3826029 and rs4779565 within *CHRNA7* and rs8136867 within *MAPK1* were significantly associated with BPD).

In complex diseases, single genetic variants contribute to disease risk only marginally. Synergic effects between SNPs can generate slightly stronger signals. This may explain why we did not find association in single SNPs analysis, while we found seemingly positive results in haplotype analysis.

The small sample size and the high number of tests performed represent major limitations of our study, strongly preventing us from drawing reliable conclusions. Though previous convincing evidence suggesting *MAPK1* in MDD,[17,18] its association with MDD in our Korean sample was small in terms of effect size and it should be therefore taken with caution. Ethnic differences prevent the generalization of the results to other populations. The genotyping methodology was not able to discriminate whether contiguous SNPs are located on the same chromosome (cis) or not (trans). Thus, haplotypes can only be inferred by a probabilistic approach.

In conclusion, the results of our study cautiously suggest an involvement of *MAPK1* in MDD in Asians, though only considering a specific combination of alleles rather than single mutations. *CACNA1C* might influence treatment outcome. However, these data are very preliminary and need to be verified.

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■ Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

SUPPLEMENTARY MATERIALS

Supplementary data are available at https://doi.org/10.9758/cpn.2019.17.3.364.

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