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Possible influence of *CREB1*, *CREBBP* and *CREM* variants on diagnosis and treatment outcome in patients with schizophrenia

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ABSTRACT

The present study explores whether some single nucleotide polymorphisms (SNPs) within *CREB1* (rs2709377 and rs6740584), *CREBBP* (rs2239317, rs2239316, rs3025702, rs130021, rs130005, rs129974 and rs9392) and *CREM* (rs1148247, rs4934735, rs12775799, rs6481941 and rs16935888) could be associated with schizophrenia (SKZ) and whether they could predict clinical outcomes in Korean in-patients treated with antipsychotics. Two-hundred twenty one in-patients suffering from SKZ and 170 psychiatrically healthy controls were genotyped for 10 SNPs within *CREB1*, *CREBBP* and *CREM*. All patients were assessed for the severity of illness at baseline and at discharge by means of the Positive and Negative Symptoms Scale (PANSS). Our findings suggest the lack of influence of SNPs under investigation in the present study on the susceptibility to SKZ and on the response to antipsychotics. However, taking into account the several limitations of our study, further research is needed to draw more definitive conclusions.

1. Introduction

Schizophrenia (SKZ) is a complex psychiatric disorder affecting approximately 1% of the population worldwide [8,22]. Both formal and molecular genetics' studies converge on suggesting that such disorder has a strong genetic etiology [12]. However, there is not yet complete consensus about the genetic variants involved [4]. An interesting candidate gene for SKZ is the one coding for the cyclic AMP-responsive element-binding protein-1 (*CREB1*), one of the messenger molecules involved in intracellular signal transduction pathways associated with a large variety of dopamine and serotonin receptor subtypes [6]. In particular, genetic variants within the promoter region of the human *CREB1* could lead to modifications of CREB expression that may influence SKZ liability and pathophysiology [6].

Of note, CREB is a member of a transcription factors' family including also CREM (cAMP response element-modulator) [9]. CREM plays a key physiological and developmental role within the hypothalamic-pituitary-gonadal axis [15]. Furthermore, both CREB and CREM can interact with CREBBP (CREB-binding protein), a protein that has an intrinsic histone acetyltransferase activity and that acts as a scaffold to stabilize additional protein interactions with the transcription complex. Interestingly, CREB–CREBBP signalling regulates long-term potentiation, a cellular mechanism that underpins memory formation [5,19]. Moreover, CATIE trial showed an association between *CREBBP* variants and SKZ [13].

On the basis of these findings, we aimed to investigate whether specific single nucleotide polymorphisms (SNPs) within genes belonging to this biological pathway, including *CREB1* (rs2709377 and rs6740584), *CREBBP* (rs2239317, rs2239316, rs3025702, rs130021, rs130005, rs129974 and rs9392) and *CREM* (rs1148247, rs4934735, rs12775799, rs6481941 and rs16935888) could be associated with the liability and response to treatment of SKZ.

2. Methods

The sample under investigation in the present study included 221 SKZ in-patients who were consecutively recruited at the

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Table 1
CREB1, *CREBBP* and *CREM* SNPs considered in this study. The relative position to the start codon is given in parenthesis. All data from www.snpper.chip.org.

Gene	SNP ID	Position	Distance	Alleles	Location	Amino acid change
CREB1	rs2709377	208393907 (−26453)	−35444	A/T	Promoter	None
CREB1	rs6740584	208429351 (8992)		C/T	Intron	None
CREBBP	rs2239317	3920740 (9178)	6745	C/G	Intron	None
CREBBP	rs2239316	3913995 (15923)	53355	A/G	Intron	None
CREBBP	rs3025702	3860640 (69278)	28169	A/G	Coding exon	313 D/?
CREBBP	rs130021	3832471 (97447)	4123	A/G	Intron	None
CREBBP	rs130005	3828348 (101570)	33056	A/G	Intron	None
CREBBP	rs129974	3795292 (134626)	20123	G/T	Coding exon	1262 I/? 1300 I/?
CREBBP	rs9392	3775169 (154749)		G/A	3′ UTR	None
CREM	rs1148247	35496946 (70183)	216	G/A	Intron	None
CREM	rs4934735	35496730 (69967)	8466	A/G	Intron	None
CREM	rs12775799	35488264 (61501)	48123	C/T	Intron	None
CREM	rs6481941	35440141 (13378)	7736	G/A	Intron	None
CREM	rs16935888	35432405 (5642)		T/C	Intron	None

Department of Psychiatry of the Catholic University of Korea College of Medicine, Seoul, Korea. The sample has been previously investigated by our group with regard to other gene variants (e.g. [14,17]). Briefly, patients were eligible for inclusion if they had a documented clinical diagnosis of SKZ according to the DSM-IV criteria [18]. All patients admitted to the hospital were assessed for the severity of illness at baseline and at discharge by means of the Positive and Negative Symptoms Scale (PANSS) [7]. A further sample of 170 Korean psychiatrically healthy subjects, who underwent the same assessment of psychiatric patients to exclude possible psychiatric disorders as well as a familiarity for 1st and 2nd degree relatives with psychiatric disorders, was also included to compare genotype and allelic frequencies between SKZ patients and psychiatrically healthy controls. The study protocol was approved by the institutional review board (approval number HC10TISI0031). All patients (18–65 years old) provided written informed consent before participating into the study.

The main outcome measures of the present study were (1) differences between genotypic and allelic frequencies in patients with SKZ as compared with healthy control subjects and (2) possible influences of the 14 SNPs mentioned above on clinical improvement as measured with the PANSS total in SKZ patients. Further outcomes of interests included improvements on PANSS subscales, on response rates and on further clinical and socio-demographical variables. Both continuous and categorical analyses were performed. In accordance with previous studies, response was a priori defined as a ≥50% symptoms' reduction from baseline to discharge [10]. Genomic DNA was extracted from blood by standard methods and quantified. The high-throughput genotyping method using pyrosequencer (Biotage AB, Sweden) was used for genotyping 14 SNPs within 3 genes under investigation (Table 1). SNPs were selected balancing criteria of coverage, possible functionality and feasibility. PCR primers (Bioneer, Daejeon, Korea) and sequencing primers (Bioneer, Daejeon, Korea) used for the pyrosequencing assay were designed by using the Pyrosequencing Assay Design Software v1 (Biotage AB, Sweden) and one primer of each primer set was biotinylated.

Statistical analyses were performed using 'Statistica' package [20]. Differences in the allelic and genetic frequencies between healthy subjects and patients with SKZ as well as effects of such variants on response rates and further categorical outcomes were calculated using the χ^2 statistics. The influence of the SNPs under investigation and continuous outcomes were calculated using the ANOVA. Clinical improvement on PANSS total scores was calculated according to the following formula:

$$\frac{\text{PANSSfinal} - \text{PANSSbaseline}}{\text{PANSSbaseline}} \times 100$$

In case of positive findings, clinical variables correlated with the outcome measures under investigation were added as covariates. Haploview 3.2 was used to generate a linkage disequilibrium (LD) map and to test for Hardy–Weinberg equilibrium (HWE) [1]. Tests for associations using multi-marker haplotypes were performed using the statistics environment "R" (http://www.R-project.org), package "haplo.score", to compare clinical and socio-demographic outcomes among different haplotypes. Permutations ($n = 10.000$) were performed to estimate the global significance of the results for all haplotypes analyses. All p-values were 2-tailed. In order to reduce the likelihood of false positive findings, statistical significance was set at the 0.006 level (approximately corresponding to the Bonferroni correction for 8 blocks of SNPs, see below). With these parameters we had a sufficient power (0.80) to detect a small–medium effect size ($\omega = 0.18$) that, as an example, corresponded to an odds ratio of 2.1 between SKZ patients and psychiatrically healthy controls and to detect a medium ($d = 0.25$) effect size for patients with SKZ carrying the GG genotype of rs129974 as compared with those carrying the TG genotype [3]. Such effects size corresponded to the possibility of detecting differences on PANSS total improvement scores of 3.2 points.

3. Results

Socio-demographic features such as gender, age and further clinical and socio-demographical variables are reported in Table 2. For control subjects only data about gender and age were collected.