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No relationship found between -1438A/G polymorphism of the serotonin 2A receptor gene (rs6311) and major depression susceptibility in a northeastern Thai population

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ABSTRACT. Several lines of evidence suggest a molecular role of -1438A/G single nucleotide polymorphism in the *5-HTR2A* gene promoter (rs6311) in regulating the expression of this gene, making rs6311 polymorphism a promising candidate for an association study. We looked for a possible association between rs6311 polymorphism and major depressive disorder (MDD) in a northeastern Thai population. We included 180 patients with MDD and 183 unrelated healthy controls in our study. Genotyping was performed using PCR-RFLP. We found no significant differences between the two groups with

regard to both genotype distributions (χ^2 = 1.32, d.f. = 2, P = 0.516) and allele frequencies (χ^2 = 0.01, d.f. = 1, P = 0.913, odds ratio = 0.96, 95% confidence interval = 0.67-1.39). Therefore, this single nucleotide polymorphism appears not to be involved in the etiology of MDD.

Key words: Major depressive disorder; Association study; Rs6311; Serotonin 2A receptor; Single nucleotide polymorphism; Thai population

INTRODUCTION

Major depressive disorder (MDD) is one of the most pressing public health issues due to its high lifetime prevalence of approximately 15% and its association with significant disability (Moussavi et al., 2007).

MDD is projected to be the second leading cause of disease burden worldwide, and the first leading cause in high-income countries for disability-adjusted life years (DALY) in 2030 (Mathers and Loncar, 2006).

Although the precise causes of MDD have not been fully elucidated, both genetic and environmental factors are acknowledged to significantly modify the risk for MDD. In particular, the total contribution of genetic factors in the origin of MDD is roughly 40% (Sullivan et al., 2000). Therefore, relevant DNA sequence variations in promising candidate genes involved in the susceptibility to MDD remain to be elucidated.

Serotonin receptor 2A (*5-HTR2A*) genetic variations have been studied as functional candidates in numerous neuropsychiatric disorders with respect to not only susceptibility, but also pharmacogenetics (Sanders-Bush et al., 2003; Norton and Owen, 2005; Serretti et al., 2007). The most extensively investigated single nucleotide polymorphisms (SNPs) of this particular gene are -1438A/G, 102T/C and 1354C/T (His452Tyr) corresponding to NCBI dbSNP cluster IDs, rs6311, rs6313 and rs6314, respectively. Functionally, no evidence has revealed the influence of rs6313 and rs6314 on transcription of the *5-HTR2A* gene. On the contrary, rs6311 has been shown to transcriptionally modulate the expression of this gene (Parsons et al., 2004; Myers et al., 2007). Consistently, *in silico* analysis has found the potential function of the rs6311 A allele due to its possession of a consensus binding site for transcription factor Th1/E47, and allele-specific binding has been reported using an electrophoretic mobility shift assay (Smith et al., 2008). Furthermore, this allele has been shown to be associated with increased 5-HTR2A receptor binding (Turecki et al., 1999). Divergences in disease susceptibility may be due to the differentially modulated density of the receptor critical for neurotransmitter mechanisms, thereby making this SNP a promising candidate for an association study.

Regarding association studies in various ethnic samples, the rs6311 A allele has been found to be associated with an increased risk for MDD in Swedish (Jansson et al., 2003) and Danish (Christiansen et al., 2007) populations, whereas the association between the rs6311 G allele and an increased risk for MDD has been reported in a Korean study (Choi et al., 2004). Nevertheless, negative associations between this SNP and MDD have been revealed in other populations including the Japanese (Ohara et al., 1998; Kishi et al., 2009), Israeli-Jewish (Frisch et al., 1999), Caucasians from France (Bonnier et al., 2002), and Finnish (Illi et al., 2009). These contradicting results may be due to a difference in ethnicities. It is known that

the frequency of the rs6311 alleles differs among ethnic groups. Most recently, the rs6311 A allele has been reported to be the major allele in healthy Thai subjects (Ronpirin et al., 2010), and this similarity has also been observed in Chinese populations (Zhang et al., 2008; Ying et al., 2009). Nevertheless, the genetic association between the rs6311 polymorphism and MDD has never been analyzed in our population. In the present study, we used a case-control study design to investigate the possible association of this SNP with susceptibility to MDD in a northeastern Thai population.

MATERIAL AND METHODS

Subjects

We successfully genotyped genomic DNA samples obtained from 180 unrelated patients with MDD (116 women and 64 men, aged 44.90 ± 12.93 years) and 183 unrelated healthy controls (126 women and 57 men, aged 42.07 ± 9.79 years). The MDD subjects were diagnosed according to DSM-IV criteria (American Psychiatric Association, 1994) by experienced psychiatrists of the Loei Rajanagarindra Psychiatric Hospital, the organization providing mental healthcare services for Loei, Nongbualamphu, and other nearby provinces of Thailand. The two groups permanently resided in the same geographic region, and were gender and age matched. The local Ethics Committee for medical experiments on human subjects approved the study, and all participants gave their written informed consent.

DNA extraction

Genomic DNA samples were isolated from whole blood using a FlexiGene DNA kit (Qiagen GmbH, Hilden, Germany).

Determination of the rs6311 polymorphism

Genotyping of the rs6311 SNP was performed by polymerase chain reaction, using specific primers (forward primer 5'- AAC CAA CTT ATT TCC TAC CAC -3' and reverse primer 5'- AAG CTG CAA GGT AGC AAC AGC -3') and subsequent restriction fragment length polymorphism with *Msp*I, as formerly addressed (Collier et al., 1997). The fragments were resolved by 3% agarose gel electrophoresis and visualized by ethidium bromide staining. Fragments containing the uncut A allele had a 468-bp band; fragments containing the G allele had two bands of 244 and 224 bp. Randomly selected DNA samples were subjected to direct sequencing to validate the genotypes.

Statistical analysis

For gender data, patients with MDD and healthy control groups were compared by the chi-square (χ^2) tests in contingency tables. Difference in age data between the two groups was examined using the unpaired Student *t*-test. Hardy-Weinberg equilibrium for the distributions of genotypes was estimated by the χ^2 test. Genotype and allele frequencies were compared between groups using the χ^2 test with Yates correction. Odds ratios (OR) and the 95% confidence

interval (CI) were calculated using EpiCalc 2000 version 1.02 (http://www.brixtonhealth.com/epicalc.html). A P value of <0.05 was considered to be significant.

RESULTS

Regarding gender and age, characteristics of patients with MDD and healthy controls were compared. There was no statistically significant difference between these two groups with respect to gender (P = 0.373). However, there was a difference in age between the two groups (P = 0.009). In particular, proportions of 30 and 40 year olds were higher in the control group, while proportions of 50 and 60 year olds were to some extent elevated in the patient group.

The genotype distributions and allele frequencies of the rs6311 SNP were determined in 363 Thai subjects, which consisted of 180 patients with MDD and 183 healthy controls (Table 1). The most common genotype in this Thai population was the AA homozygote; the number of GG homozygotes was small. The genotype distributions of the rs6311 SNP were in Hardy-Weinberg equilibrium for healthy controls ($\chi^2 = 3.12$, d.f. = 1, P = 0.077).

Table 1. Genotype distributions and allele frequencies of the rs6311 single nucleotide polymorphism in patients with major depressive disorder (MDD) and healthy controls.

Variables	Genotype (%)					Allele (%)				
	AA	AG	GG	χ^2 d.f.=2	P	A	G	χ^2, d.f.=1	P	OR (95%CI)
MDD patients (N = 180)	110 (61.11%)	69 (38.33%)	1 (0.56%)			289 (80.28%)	71 (19.72%)			
Healthy controls (N = 183)	116 (63.39%)	64 (34.97%)	3 (1.64%)	1.32	0.516	296 (80.87%)	70 (19.13%)	0.01	0.913	0.96 (0.67-1.39)

d.f. = degrees of freedom; OR = odds ratio; CI = confidence interval.

There were no significant differences between patients with MDD and healthy controls with regard to both genotype distributions ($\chi^2 = 1.32$, d.f. = 2, P = 0.516) and allele frequencies ($\chi^2 = 0.01$, d.f. = 1, P = 0.913, OR = 0.96 [95%CI = 0.67-1.39]).

DISCUSSION

Due to the experimentally proven significance of the rs6311 SNP in the transcriptional regulation of *5-HTR2A* gene expression, this SNP has attracted much attention for not only genetic association studies, but also for pharmacogenetics studies with regard to numerous neuropsychiatric diseases, such as attention deficit, hyperactivity disorder, Alzhiemer's disease, schizophrenia, and mood and anxiety disorders (Sanders-Bush et al., 2003; Norton and Owen, 2005; Serretti et al., 2007). It has been reported that activation of *5-HTR2A* influences multiple cell functions depending on the second messenger cascade (Duman et al., 1997; Vaidya et al., 1997) since it is coupled with protein kinase C via increased phosphoinositide breakdown like all *5-HTR2* receptors (Saxena, 1995).

To the best of our knowledge, this is the first study to investigate a potential association between such functionally relevant genetic variation in the promoter of the gene encoding *5-HTR2A* (rs6311) and MDD in a Thai population. In the present investigation, we found no overall differences in genotype distributions and allele frequencies when patients with MDD were compared with healthy controls. It is recognized that the frequency of the rs6311 alleles

differs among ethnic groups. In accordance with a previous study (Ronpirin et al., 2010), the rs6311 A allele was found to be the major allele in Thais. We found a higher percentage of the rs6311 A allele, about 80%, in healthy Thai subjects, while the frequency of this allele reported by Ronpirin et al. (2010) was only 74%. The difference in allele frequencies between the two studies may be due to healthy controls from dissimilar demographic regions analyzed. In our study, we collected blood samples from healthy controls, who only resided in the northeastern part of Thailand, whereas Ronpirin et al. (2010) recruited healthy individuals nationwide. Like the Chinese, the rs6311 A allele has been proven to be the major allele (Zhang et al., 2008; Ying et al., 2009). Functionally, this SNP has been found to be negatively associated with suicidal behavior in Chinese psychiatric patients (Zhang et al., 2008). Another functional SNP in the *5-HTR2A* gene, rs6313, has also been reported to be negatively associated with suicidal ideation in a Chinese population (Wang et al., 2009).

Regarding the study groups recruited in this study, both patient and control groups resided in a similar demographic region, and their psychosocial backgrounds were comparable, supporting the weight given to this investigation. Regarding a point of concern, the observed difference in age between the two groups in the present study may have influenced the outcome in the association test as previously discussed (Angunsri et al., 2009). Nevertheless, it is unlikely that such an age difference could significantly alter the result of this study. It is worth noting that we successfully carried out the study by taking the supreme precaution to prevent misgenotyping and misinterpretation. Particularly, there were certain case studies with regard to this issue. First, we have discovered specific misgenotyping results of SNP in one of the important genes of the dopaminergic system, dopamine receptor D1 gene -48A/G polymorphism (Tencomnao and Boonmalert, 2009). Second, our group has recently reported mismatches between the two SNP numbers (rs6311 and rs6313) previously published elsewhere (Tencomnao, 2010).

In conclusion, we found no evidence that the rs6311 polymorphism is associated with an altered risk of MDD in a northeastern Thai population. Nonetheless, other polymorphisms in this gene may play a role in the etiology of MDD; exploring their functional significance would be useful.

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