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Family- and population-based association studies of monoamine oxidase A and autism spectrum disorders in Korean

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ABSTRACT

Monoamine oxidase A gene (*MAOA*) has been thought to be a candidate gene implicated in autism spectrum disorder (ASD). This study evaluates the relationship between ASDs and *MAOA* markers (i.e., uVNTR and four single nucleotide polymorphisms (SNPs)) in 151 Korean family trios with children diagnosed with ASDs, and 193 unrelated Korean controls. The result of case–control global haplotype analysis also showed a statistically significant difference in haplotype frequencies between ASD patients and controls (male d.f. = 5, $p < 0.001$; female d.f. = 7, $p < 0.001$). With the specific haplotype analyses, the frequencies of the most frequent haplotype (AGG) with three SNPs (rs5906883 + rs1137070 + rs3027407) in ASD showed significant statistical differences between ASD patients and controls in both the male and female groups (d.f. = 1, male $p = 0.001$, female $p < 0.001$). In a family-based association test (FBAT) analysis, it was observed that, in the dominant model, a three-repeat allele of a MAOA-uVNTR marker was preferentially transmitted in ASDs ($Z = 2.213$, $p = 0.027$). Moreover, in the global haplotype analysis, the statistically significant evidence of associations with ASD were demonstrated in additive and dominant models (additive $\chi^2 = 11.349$, d.f. = 2, $p = 0.003$; dominant $\chi^2 = 6.198$, d.f. = 2, $p = 0.045$).

1. Introduction

Autism spectrum disorder (ASD) [Mendelian Inheritance in Man (MIM) 209850] is a neuropsychiatric developmental disorder characterized by impairments in social communication and a preference for repetitive and solitary interests and behaviors (American Psychiatric Association, 1994). The population prevalence of autism is known to be approximately 15–20 in 10,000, and that of ASD is 60 in 10,000 children. Worldwide, the male-to-female ratio of ASD is approximately 4:1 (Chakrabarti and Fombonne, 2005). Several twin and family studies have demonstrated that genetic factors contribute to ASD, and it is known that both autism and ASD are highly heritable, although the exact mode of transmission is not known. Previous studies suggest that ASD is a complex genetic disorder that most likely results from the interaction of multiple genetic and environmental factors (Yonan et al., 2003).

Recently, there have been a number of molecular genetic studies aimed at identifying a candidate gene or the genes responsible for the development of ASD. Previous linkage analysis studies have found evidence for autism loci on chromosomes; regions implicated by multiple studies include 1p, 5q, 7q, 15q, 16q, 17q, 19q, and Xq (Klauck, 2006). The high male-to-female ratio among affected children suggests that an X-linked locus could be central to autism or related pervasive development disorders (PDD) (Hallmayer et al., 1996).

In examining the various etiologies of ASD, a common neurochemical mechanism cannot be denied as a contributing

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factor. The catecholamines and serotonin are involved in mood, arousal, rewards, and neural developments. Blood serotonin is known to be correlated with verbal intelligence quotient (IQ) and autism severity (Cook et al., 1990; Kuperman et al., 1987). Monoamine oxidase A (MAOA) is an enzyme that maintains a homeostatic level across neurotransmitters (i.e., serotonin, dopamine, and norepinephrine). Therefore, *MAOA* has been thought to be a candidate gene implicated in ASD, and it has been mapped to chromosome Xq11.23–Xq11.4. A 30-bp repeat polymorphism, located 1.2 kb upstream of the *MAOA* coding sequences (MAOA-uVNTR), has been extensively studied. An in vitro expression study has revealed that the number of repeats determines the transcriptional level of the gene. There have been several studies into an association between the MAOA-uVNTR and various human behaviors such as addictive behavior, aggression, and impulsive traits (Gerra et al., 2004; Samochowiec et al., 2004; Yu et al., 2005). Yirmiya et al. (2002) examined the association between MAOA-uVNTR polymorphism and autism with 49 autism families using a transmission disequilibrium test (TDT) and a case–control study with a control group of 108 normal male subjects. Although there was a trend of association between IQ in the proband and polymorphism, they did not find any association in TDT and case–control design. Further, Cohen et al. (2003) report that functional MAOA-uVNTR alleles may act as a genetic modifier of the severity of autism in males; however, there have been few studies into the association between common single nucleotide polymorphisms (SNPs) in *MAOA* and ASD.

The objective of this study is to evaluate the association between ASD and common polymorphisms (SNPs and MAOA-uVNTR) of the MAOA gene in the Korean population.

2. Materials and methods

Subjects were recruited from a family-based genetic association study of ASD conducted by the same research group. Subject ascertainment and diagnostic methods have previously been described (Cho et al., 2007; Kim et al., 2007; Yoo et al., 2008); briefly, the ASD proband were diagnosed using the Autism Diagnostic Interview-Revised (ADI-R) (Yoo, 2007) and the Korean version of the Autism Diagnostic Observation Schedule (ADOS) (Yoo and Kwak, 2007), together with the best estimates of two board-certified child psychiatrists. Subjects diagnosed with or strongly suspected of having neurofibromatosis, tuberous sclerosis, any kind of metabolic encephalopathy, and known chromosomal abnormalities were excluded. None were diagnosed with clinically significant partial seizure disorder. The present study included 151 complete trios, comprising patients with ASD (79.9 ± 35.6 months, 86.1% male, 87.4% autism, 13.5% PDD-NOS, and 1.6% Asperger's disorder) and their biological parents. The psychological properties were fairly similar, as described previously (Kim et al., 2007).

The controls comprised 193 apparently healthy sex-matched, unrelated Koreans who had visited a public health center to evaluate their health status; they did not have psychiatric and physical diseases or a personal or familial history of psychiatric or neurological illness, and their mean age was 40.1 years (range: 23–69 years); 164 (85.0%) were males and 29 (15.0%) were females. We obtained written informed consent of all parents/primary caregivers and participants, and this study was approved by the institutional review boards of the participating institutions.

DNA was extracted from the blood of all subjects using the G-spin Genomic DNA Extraction Kit (Intron, Daejeon, Korea). MAOA-uVNTR was genotyped by polymerase chain reaction (PCR) amplification, as described in Sabol et al. (1998). Through the Entrez SNP database, we selected four SNPs (i.e., rs5906883, rs1800464, rs1137070, and rs3027407) of *MAOA* with relatively high heterozygosity. Individual SNPs were genotyped by the TaqMan genotyping assay. The PCR primers and probe sets for this assay were obtained by Assays-On-Demand™. TaqMan genotyping was performed according to manufacturer's recommendations (Applied Biosystems, Seoul, Korea).

We separated subjects by sex into case and control groups. Comparisons of alleles, genotypes, and haplotype frequencies between cases and controls were performed with a Chi-square test. Odds ratios (ORs) with 95% confidence intervals were estimated for the effects of high-frequency alleles and haplotypes in the case group. The tests for females were conducted using SNPAlyze 5.0.4 (Dynacom, Chiba, Japan). In male subjects, we calculated linkage disequilibrium coefficient (D') by a 2LD program (Zhao, 2004). The results were considered significant for markers and haplotypes with p-values less than 0.05.

A transmission disequilibrium test (TDT) of all polymorphisms was examined using the FBAT 2.0.2. In families with two affected individuals with research diagnoses of ASD, a single, randomly selected affected individual was included in the TDT analysis. To calculate the power of our TDT and case–control analysis, the genetic power calculator program was used (http://pngu.mgh.harvard.edu/~purcell/gpc/).

3. Results

We checked the Mendelian inheritance of genotypes within the trios: two Mendelian inheritance errors were found. These two trios were excluded from the data set in this TDT and haplotype analysis. Of the four SNPs of the MAOA gene, rs1800464 was not polymorphic. The genotype distribution of all polymorphisms did not deviate from what was expected, on the basis of the Hardy–Weinberg equilibrium in female subjects.

In case–control association studies using the allelic frequencies of male and female groups and the genotype frequencies of the female groups, we were also unable to detect any significant result of polymorphisms between controls and ASD patients. However, linkage disequilibrium (LD) analysis demonstrated the presence of LD between all polymorphisms ($D' > 0.5$) in the male subjects. In particular, we observed higher D' values among three SNPs in ASD patients than those of the controls (Table 1). The result of case–control global haplotype analysis with three SNP polymorphisms caused by weak LD values between uVNTR and another three SNPs, showed a statistically significant difference in haplotype frequencies between ASD patients and controls (male d.f. = 5, $p < 0.001$;

Table 1
Allele frequencies and linkage disequilibrium between polymorphisms of MAOA gene in autism spectrum disorders and controls.

Polymorphism (allele)	Male		Female	
	ASD	Control	ASD	Control
VNTR (3)	0.624	0.526	0.545	0.632
rs5906883 (A)	0.632	0.526	0.523	0.528
rs1137070 (G)	0.608	0.526	0.5	0.431
rs3027407 (G)	0.616	0.526	0.477	0.542

Linkage disequilibrium (D') matrix in case–control data (ASDs/controls)

Male			
	uVNTR	rs5906883	rs1137070
rs5906883	0.652/0.531		
rs1137070	0.615/0.820	0.964/0.507	
rs3027407	0.620/0.844	0.965/0.507	1.000/0.974
Female			
	uVNTR	rs5906883	rs1137070
rs5906883	0.898/0.187		
rs1137070	0.890/0.253	1.000/0.670	
rs3027407	0.787/0.073	0.906/0.618	1.000/1.000