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Association of the Catechol-*o*-Methyltransferase Gene Polymorphisms with Korean Autism Spectrum Disorders

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This study evaluated the family-based genetic association between autism spectrum disorders (ASDs) and 5 single-nucleotide polymorphisms (SNPs) in the catechol-*o*-methyltransferase gene (*COMT*), which was found among 151 Korean ASDs family trios (dominant model Z = 2.598, $P = 0.009$, $P_{FDR} = 0.045$). We found a statistically significant allele transmission or association in terms of the rs6269 SNP in the ASDs trios. Moreover, in the haplotype analysis, the haplotypes with rs6269 demonstrated significant evidence of an association with ASDs (additive model rs6269-rs4818-rs4680-rs769224 haplotype $P = 0.004$, $P_{FDR} = 0.040$). Thus, an association may exist between the variants of the *COMT* gene and the occurrence of ASDs in Koreans.

Key Words: Autism Spectrum Disorders (ASD); Polymorphisms, Single Nucleotide (SNPs); Catechol-O-Methyltransferase Gene (*COMT*); Family-Based Association Study

The dopaminergic system is known to affect a wide range of behaviors and brain functions. Catechol-*o*-methyltransferase (COMT) has been implicated as playing a role in a variety of psychiatric symptoms and diseases, including phobic anxiety, obsessive-compulsive disorder, schizophrenia, and attention-deficit hyperactivity disorder (1, 2). Presuming that autism spectrum disorders (ASDs) are associated with a high level of anxiety, genetic overlap with schizophrenia, and a high level of sexual difference, it can be hypothesized that COMT may be one of the contributing factors in the pathogenesis of ASDs (2, 3). Of the several single nucleotide polymorphisms (SNPs) of *COMT*, rs4680 is a common and well-known normal variant. Chen et al. (4) revealed that rs4680 significantly affects the protein abundance and enzyme activity in postmortem human prefrontal cortex tissue.

Based on this concept, several studies investigating the genetic association between *COMT* polymorphisms and ASDs have been conducted. Although James et al. (5) reported a genetic association with polymorphism rs4680 in a case-controlled setting, some family-based association tests failed to identify a linkage/association with this SNP (6, 7). In this study, we conducted an association study by using some other common SNPs of the *COMT* gene in Korean families with ASDs to confirm the genetic association with different populations.

This study was performed with 151 Korean complete ASDs trios comprising patients with ASDs (age, 79.9 ± 35.6 months; male, 86.1%; patients with autism, 87.4%; patients with pervasive developmental disorder not otherwise specified, 13.5%; and patients with Asperger's disorder, 1.6%) and their biological parents. The ASDs probands were diagnosed using the Korean version of Autism Diagnostic Interview-Revised (ADI-R) and the Korean version of the Autism Diagnostic Observation Schedule (ADOS), together with an evaluation conducted by 2 board-certified child psychiatrists. The subject ascertainment and diagnostic methods used have been previously described (8). The study protocol was approved by the institutional review board of Eulji University (IRB No. EU 08-06).

Genomic DNA from blood samples was prepared using a G-

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spin Genomic DNA Extraction Kit (Intron, Daejeon, Korea). The structures of the candidate genes were determined using the Entrez SNP database (http://www.ncbi.nlm.nih.gov/) and a publicly available genotype database for Asian populations from the International HapMap Project (http://www.hapmap.org). The SNPs located in the coding region and 5´ and 3´ regions were selected (minor allele frequencies of greater than 0.05 in the Chinese and Japanese populations). Five SNPs in the *COMT* gene (rs6269, rs4818, rs4680, rs769224, and rs165728) were selected for the study and genotyped using the GoldenGate™ Assay (Illumina, San Diego, CA, USA).

The Mendelian inheritance error and Hardy-Weinberg equilibrium for each pair of SNPs were evaluated with the transmission disequilibrium test (TDT) method in Haploview v3.2 (http://www.broad.mit.edu/mpg/haploview). Family-based association tests for each individual polymorphism and haplotype were performed using the Family-based Association Test (FBAT) program package (v2.0.2). HBAT, the haplotype version of the FBAT program, was used to identify haplotypes with a greater than 5% frequency of association with ASDs. Haplotype tests were performed using permutations (n = 100,000 cycles) with the HBAT, Monte Carlo option. A quantitative transmission disequilibrium test was also performed with the FBAT. For the quantitative behavioral scales, we explored 3 domain scores (qualitative abnormalities in reciprocal social interaction; qualitative abnormalities in communication; and restricted, repetitive, and stereotypical pattern of behavior) and 12 subdomain scores listed in the ADI-R (9). Both single-marker and haplotype testing were carried out for the affection status and each quantitative trait. The power calculation for the association test and samples was performed using the TDT for discrete traits, available at the Genetic Power Calculator web site (http://pngu.mgh.harvard.edu/~purcell/gpc/). A P value of less than 0.05 was considered statistically significant. We applied the false-discovery rate (FDR) procedure, which was proposed by Benjamini and Hochberg (10) for handling multiple comparison problems. FDR corrections were performed separately for single markers and haplotypes. FDR-corrected P values (P_{FDR}) of less than 0.05 were considered to be significant.

In the linkage disequilibrium test for each pair of markers, the 5 SNPs were in weak-to-strong linkage disequilibrium with respect to one another (0.49 < D´ < 1.00). In the biallelic mode, we obtained statistically significant results for the rs6269 polymorphism in the additive and dominant/recessive models (G allele, additive: Z = 2.020, P = 0.043, P_{FDR} = 0.215; dominant: Z = 2.598, P = 0.009, P_{FDR} = 0.045). In the multiallelic mode, no statistically significant results were obtained for rs6269 in the additive and dominant models, after multiple-testing correction (additive: df = 1, P = 0.043, P_{FDR} = 0.215; dominant: df = 2, P = 0.033, P_{FDR} = 0.165) (Table 1).

Significant P values were observed for some haplotypes containing markers for *COMT*. A total of 16 haplotypes were observed with the 5 SNPs, and haplotypes with a frequency of greater than 0.05 were selected. We conducted haplotype analyses by using the sliding windows methods to identify specific haplotypes that were significant in the multiallelic mode. The haplotypes, including the rs6269 SNP, revealed statistically significant associations in the additive models (rs6269-rs4818 haplotype: P = 0.011, P_{FDR} = 0.055; rs6269-rs4818-rs4680 haplotype: P = 0.026, P_{FDR} = 0.087; rs6269-rs4818-rs4680-rs769224 ha-

Table 1. FBAT analyses of markers of *COMT* gene in possible mode and models

Biallelic mode Marker	Allele	Freq.	Additive			Dominant			Recessive		
			N	Z	*P*	N	Z	*P*	N	Z	*P*
rs6269	A	0.678	72	-2.020	0.043	38	-0.178	0.859	60	-2.598	0.009
	G	0.322	72	2.020	0.043	60	2.598	0.009	38	0.178	0.859
rs4818	C	0.348	95	1.449	0.147	78	0.296	0.767	44	2.212	0.027
	G	0.652	95	-1.449	0.147	44	-2.212	0.027	78	-0.296	0.767
rs4680	A	0.283	103	-0.351	0.726	92	-0.379	0.705	38	-0.089	0.929
	G	0.717	103	0.351	0.726	38	0.089	0.929	92	0.379	0.705
rs769224	A	0.056	29	0.730	0.465	29	0.839	0.401	1	-0.577	0.564
	G	0.944	29	-0.730	0.465	1	0.577	0.564	29	-0.839	0.401
rs165728	A	0.609	113	0.241	0.810	60	-0.499	0.618	95	0.708	0.479
	G	0.391	113	-0.241	0.810	95	-0.708	0.479	60	0.635	0.618

Multi-allelic mode Model			Additive			Dominant/Recessive		
Markers	Number of allele	N	*df*	χ^2	*P*	*df*	χ^2	*P*
rs6269		72		4.082	0.043		6.803	0.033
rs4818		95		2.098	0.147		4.893	0.087
rs4680	2	104	1	0.123	0.726	2	0.145	0.930
rs769224		29		0.533	0.465		1.093	0.579
rs165728		113		0.058	0.810		0.893	0.640

Trait affection; offset 0.000; specifying minimum number of informative families 0; mininum frequency 0.050; The Z and χ^2 tests produced by "FBAT" are large sample tests, based on the number of informative families (*N*). *df*, degree of freedom; χ^2, χ^2 statistic.

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plotype: $P = 0.004$, $P_{FDR} = 0.040$; rs6269-rs4818-rs4680-rs769224-rs165728 haplotype: $P = 0.044$, $P_{FDR} = 0.110$). Moreover, we could confirm the results for these haplotypes in the biallelic mode as well. The haplotype formed by 4 SNPs (rs6269 [A], rs4818 [G], rs4680 [G], and rs769224 [A]) revealed consistent statistically significant associations according to the additive (Z = -2.784, $P = 0.005$, $P_{FDR} = 0.025$) and the dominant (Z = -2.677, $P = 0.007$, $P_{FDR} = 0.035$) models.

In the quantitative trait analysis using the ADI-R diagnostic algorithm scores, no significant results were observed with the rs6269 SNP. However, significant associations were observed between rs4680 and the BV total scores (qualitative abnormalities in communication trait for verbal subjects) in the dominant/recessive models ($df = 2$, $P = 0.038$). rs165728 was associated with the A1 trait (failure to use nonverbal behaviors to regulate social interaction; additive: $df = 1$, $P = 0.032$), and rs4818 was associated with the C2 (apparent compulsive adherence to nonfunctional routines or rituals; additive: $df = 1$, $P = 0.037$) and C4 (preoccupations with parts of objects or nonfunctional elements of material; additive: $df = 1$, $P = 0.023$) traits. In addition, the rs6269-rs4818-rs4680-rs769224 haplotypes were associated with the B4 trait (lack of varied spontaneous make-believe or social imitative play; additive: $df = 7$, $P = 0.041$; dominant: $df = 7$, $P = 0.034$), although these quantitative trait analysis results lost significance after applying FDR correction. In the power calculation for the markers, we employed the additive model with high-risk allele frequency, a D' value of 1, and a prevalence of 0.006. In the TDT module, the values of power were 0.078 and 0.515, respectively.

This family-based association study supports a possible association between rs6269 and other SNPs in the linkage disequilibrium (LD) region of the *COMT* gene for ASDs-affected Korean individuals. Although several studies have described the association between rs6269 and cognitive functions as well as several disorders such as schizophrenia and major depressive disorder, to the best of our knowledge, this study is the first to report an association between rs6269 and ASDs (11, 12). Because the shorter form of the *COMT* transcript may be regulated by a distal *P2* promoter region in which rs6269 is located (13), rs6269 might be involved in gene expression regulation.

The effects of rs4680 on *COMT* have been extensively studied in typical and neuropsychiatric populations. From the quantitative trait analysis in this study, it was revealed that rs4680 has a marginally significant association with the ADI-R-listed qualitative abnormalities in communication trait for verbal subjects. James et al. (5) reported the association between ASDs and rs4680, with a 1.74-fold increased susceptibility. The case control analysis with our sample and the published Korean control population did not indicate a significant association (14) (data not shown). These results may be a consequence of ethnic differences, because the Asian and Caucasian populations have different minor allele frequencies.

In this study, only ADI-R diagnostic algorithms were used to analyze the quantitative traits of the samples. Although we observed some relevance between SNPs in *COMT* and specific traits, the rs6269 SNP did not have any association with the quantitative traits. Therefore, it might be helpful to use other quantitative traits of ASDs, assessed using biological or psychological tools for quantitative trait analysis. In addition, although we selected common SNPs of the population to get more informative families for this family-based analysis, this selection had some limitations in covering all of the *COMT* variants. Although the rs6269 SNP was associated with ASDs in the Koreans in this study, the analysis involved a relatively small sample and showed deficits in power with the power analysis. For the quantitative trait test, it would appear that the sample size did not confer enough power to the analysis performed. Moreover, the significant associations did not survive after multiple-testing corrections. Therefore, this study needs to be replicated and verified with a larger sample size and other ethnic groups that have enough clinical data on the cognitive and executive functions for an effective quantitative trait analysis. In addition, for the hypothesized "common disease/rare variant model," a genetic analysis needs to be conducted with several rare variants of the gene (15).

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