Association of the Catechol-\(\alpha\)-Methyltransferase Gene Polymorphisms with Korean Autism Spectrum Disorders

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The dopaminergic system is known to affect a wide range of behaviors and brain functions. Catechol-\(\alpha\)-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-controll-
ed 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-
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 Haplovie v3.2 (http://www.broad.mit.edu/mpg/haplovie). 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 = 0.443, 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} = 0.067; rs6269-rs4818-rs4680-rs769224

| Table 1. FBAT analyses of markers of COMT gene in possible mode and models |
|---------------------|----------------|----------------|----------------|----------------|----------------|
| **Biallelic mode**  | **Marker**    | **Allele** | **Freq.** | **Additive** | **Dominant** | **Recessive** |
| **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            | 0.339         | 0.401         | 1             | -0.577        | 0.564         |
|                     | G             | 0.944        | 29            | -0.730        | 0.465         | 1             | 0.577         | 0.564         | 29            | -0.339        | 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** | **Markers** | **Number of allele** | **N** | df | χ² | P |
|------------------------|-----------|-------------|----------------------|-------|----|----|---|
| rs6269                 |           |             | 72                   | 4.082 | 0.043 | 6.803 | 0.033 |
| rs4818                 |           |             | 95                   | 2.098 | 0.147 | 4.893 | 0.087 |
| rs4680                 | 2         | 104         | 1.023                | 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 |

Traits affection; offset 0.000; specifying minimum number of informative families 0; minimum frequency 0.050; The Z and χ² tests produced by “FBAT” are large sample tests, based on the number of informative families (N); df, degree of freedom; χ², χ² statistic.
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).

REFERENCES

1. Tunbridge EM, Harrison PJ. Importance of the COMT gene for sex differences in brain function and predisposition to psychiatric disorders. Curr Top Behav Neurosci 2011; 8: 119–40.
2. Gadow KD, Roohi J, DeVincent CJ, Kirsch S, Hatchwell E. Association of COMT (Val158Met) and BDNF (Val66Met) gene polymorphisms with anxiety, ADHD and tics in children with autism spectrum disorder. J Autism Dev Disord 2009; 39: 1542–51.
3. Rzhetsky A, Wajngurt D, Park N, Zheng T. Probing genetic overlap among complex human phenotypes. Proc Natl Acad Sci U S A 2007; 104: 11694-9.
4. Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kola-chana BS, Hyde TM, Herman MM, Apud J, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 2004; 75: 807–21.
5. James SJ, Mehnyk S, Jernigan S, Cleves MA, Halsted CH, Wong DH, Cutler P, Bock K, Boris M, Bradstreet JJ, et al. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. Am J Med Genet B Neuropsychiatr Genet 2006; 141B: 947-56.
6. Yirmiyia N, Plilowsky T, Nemarov L, Arbelle S, Feinsilver T, Fried I, Ebstein RP. Evidence for an association with the serotonin transporter promoter region polymorphism and autism. Am J Med Genet 2001; 105: 381-6.
7. Anderson BM, Schnetz-Boutaud N, Bartlett J, Wright HH, Abramson RK, Cuccaro ML, Gilbert JR, Pericak-Vance MA, Haines JL. Examination of association to autism of common genetic variation with genes related to dopamine. Autism Res 2008; 1: 364-9.
8. Yang SY, Cho SC, Yoo HJ, Cho IH, Park M, Kim BN, Kim JW, Shin MS,
Park TW, Son JW, et al. Association study between single nucleotide polymorphisms in promoter region of AVPR1A and Korean autism spectrum disorders. Neurosci Lett 2010; 479: 197-200.

9. Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord 1994; 24: 659-85.

10. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B (Methodological) 1995; 57: 289-300.

11. Kocabas NA, Faghel C, Barreto M, Kasper S, Linotte S, Mendlewicz J, Noro M, Oswald P, Souery D, Zohar J, et al. The impact of catechol-O-methyltransferase SNPs and haplotypes on treatment response phenotypes in major depressive disorder: a case-control association study. Int Clin Psychopharmacol 2010; 25: 218-27.

12. Shifman S, Bronstein M, Sternfeld M, Pisanté-Shalom A, Lev-Lehman E, Weizman A, Reznik I, Spivak B, Grisaru N, Karp L, et al. A highly significant association between a COMT haplotype and schizophrenia. Am J Hum Genet 2002; 71: 1296-302.

13. Zhang J, Ji Y, Moon I, Pellemounter LL, Ezequel Salavaggione O, Wu Y, Jenkins GD, Batzler AJ, Schaid DJ, Weinshilboum RM. Catechol O-methyltransferase pharmacogenomics: human liver genotype-phenotype correlation and proximal promoter studies. Pharmacogenet Genomics 2009; 19: 577-87.

14. Lee SG, Joo Y, Kim B, Chung S, Kim HL, Lee I, Choi B, Kim C, Song K. Association of Ala72Ser polymorphism with COMT enzyme activity and the risk of schizophrenia in Koreans. Hum Genet 2005; 116: 319-28.

15. Buxbaum JD. Multiple rare variants in the etiology of autism spectrum disorders. Dialogues Clin Neurosci 2009; 11: 35-43.