

Microsatellite Marker in Gamma - Aminobutyric Acid - A Receptor Beta 3 Subunit Gene and Autism Spectrum Disorders in Korean Trios

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This study aimed to identify the association between gamma-aminobutyric acid-A (GABA-A) receptor subunit $\beta 3$ (GABRB3) gene and autism spectrum disorders (ASD) in Korea. Fifty-eight children with ASD [47 boys (81.0%), 5.5 \pm 4.1 years old], 46 family trios, and 86 healthy control subjects [71 males (82.6%), 33.6 \pm 9.3 years old] were recruited. Transmission disequilibrium test revealed that, 183 bp long allele in GABRB3 gene was preferentially transmitted in families with ASD ($p = 0.025$), whereas a population-based case-control study, however, showed no association between ASD and GABRB3 microsatellite polymorphism. Our data provide preliminary evidence that GABRB3 gene is associated with ASD in Korea.

Key Words : Autism spectrum disorders; genetics; transmission disequilibrium test; microsatellite; gamma-aminobutyric acid-A receptor subunit $\beta 3$.

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INTRODUCTION

Autism spectrum disorders (ASD) are childhood-onset neurodevelopmental disorders characterized by impairments in social interaction, communication, and stereotypic behaviors. Genetic factors may contribute to the pathogenesis of ASD,^{1,2} and diverse biological studies show that gamma-aminobutyric acid (GABA) is associated with ASD.³⁻⁷ Cook et al.⁸ initially presented evidence of linkage disequilibrium in the GABA type-A receptor subunit gene in chromosome 15q11-13 which includes the Prader-Willi and Angelman syndrome critical region (PWACR). Since then, several studies have replicated the positive results on the association of GABRB3 gene with autistic disorder.⁹⁻¹¹ In contrast, however, negative results have also been reported.^{12,13} Several researchers reported the association of a microsatellite marker, simply known as GABRB3, 3' to the GABRB3 gene, with autistic disorder.^{10,11} Since no genetic studies have so far been conducted in Korean subjects with ASD, we employed both family-based and population-based analyses to investigate the association of GABRB3 microsatellite marker of the GABRB3 gene.

Fifty-eight Korean children with ASD [47 boys (81.0%), mean age, 5.5 \pm 4.1 years old] were enrolled from the outpatient clinic at a general hospital in Seoul, Korea. Biological parents of 46 patients among those 58 patients with ASD were also recruited. Eighty-six healthy control subjects [71 males (82.6%), mean age, 33.6 \pm 9.3 years old] were also recruited through advertisements in the local newspapers. The study protocol was approved by the Institutional Review Boards. After a complete description of the study to participants, written informed consent was obtained.

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Table 1. Genotype Distributions of GABRB3 Polymorphism and the Results of Transmission Disequilibrium Test (TDT)

(A) Genotype distributions of GABRB3 polymorphism				
	Genotype distributions, N (%)			<i>p</i> value
	183 bp/183 bp	183 bp/Others	Others/Others	
ASD (n = 58)	32 (55.2)	20 (34.5)	6 (10.3)	0.89
Control (n = 86)	44 (51.2)	32 (37.2)	10 (11.6)	

(B) The results of transmission disequilibrium test				
		Transmitted (N)		<i>p</i> value*
		183 bp allele	Others	
Not transmitted (n)	183 bp allele	38	15	0.025
	Others	30	9	

GABRB3, gamma-aminobutyric acid A receptor, subunit β 3; ASD, autism spectrum disorders; Others, all alleles except 183 bp allele. **p* value for TDT test, McNemar χ^2 value = 5.000, *df* = 1.

A trained child psychiatrist diagnosed patients according to criteria in the Diagnostic and Statistical Manual of Mental Disorder-fourth edition (DSM-IV). Twenty children (34.5%) were diagnosed with autistic disorders, 5 (8.6%) with Asperger's disorder, and 33 (56.9%) with pervasive developmental disorder not otherwise specified (PDD NOS). To assess healthy control subjects within the normal range of intelligence, the Structured Clinical Interview for DSM-IV¹⁴ and the Wechsler Adult Intelligence Scale-Revised¹⁵ were administered. Children with chromosomal abnormalities or a history of definite head trauma were excluded.

Genomic DNA was extracted from peripheral leukocytes of subjects using a standard phenol/chloroform method (Invitrogen Easy-DNA™ Kit, Boehringer Mannheim, San Diego, California, USA), according to the manufacturer's instructions. Genotyping was performed with an investigator blinded to the status of the probands and family information. Polymerase chain reaction (PCR) was performed with sense primer 5'-CTCTTGTTCTGTTGCTTCAATACAC-3' and anti-sense primers 5'-CACTGTGCTAGTAGATTCAGCTC-3' for analyzing the GABRB3 marker located approximately 60 kb beyond the 3' end of the GABRB3 gene.¹⁶ The amplified products were analyzed by electrophoresis on 8% acrylamide-bis (19 : 1) 40% urea sequencing type gel on which subject samples were loaded.

Transmission disequilibrium test (TDT) was applied to analyze the preferential intergenerational transmission of GABRB3 microsatellite in 46 complete trios. We used the χ^2 test for comparison of allele and genotype frequencies between probands and healthy controls. Statistical significance was defined at an alpha of < 0.05, two-tailed. All analyses of genotype distribution were performed using the SNP Alyze software package V 5.0 (Dynacom Co. Yokohama, Japan).

Nine alleles (179, 181, 183, 185, 187, 191, 193, 195, 197 bp long) were observed in Korean subjects: the allele with 183 bp long was the most frequent allele (72.4%) in children with

ASD, while the other eight alleles had frequencies less than 10%. When we divided the alleles into two subclasses (allele with 183 bp long and others), no deviation from Hardy-Weinberg equilibrium was seen in ASD ($p = 0.30$) and normal controls ($p = 0.27$). TDT result disclosed that allele with 183 bp long in GABRB3 was significantly preferentially transmitted in families with autism spectrum disorders (McNemar χ^2 value = 5.000, *df* = 1, $p = 0.025$). However, no differences were observed between the allele and genotype frequencies of ASD probands and healthy control subjects (Table 1).

To our best knowledge, this is the first report on the association between the GABRB3 microsatellite polymorphism of GABRB3 gene and ASD in Korea, although Kim et al.¹⁷ have reported the association of single nucleotide polymorphism (SNP) of GABRB3 gene with ASD in Korean trios. The allele frequencies of GABRB3 microsatellite markers in this Korean population differed from those of a previous foreign study.^{10,11} Martin et al.¹¹ reported 187 bp long allele that was transmitted less often than expected, whereas another two alleles, 191 and 197 bp long alleles, were transmitted with increased frequencies. The allele with 191 bp long was also reported to be overtransmitted to the children in Curran et al.¹⁰ study. However, we observed that 183 bp long allele was overtransmitted to children with ASD when we considered 8 other alleles (179, 181, 185, 187, 191, 193, 195, 197 bp long) together as one category of rare alleles because of the less than 10% of allele frequencies. The data indicated clear ethnic differences in polymorphism of GABRB3 microsatellite marker of Korean subjects.

In the present study, both family-based and population-based association studies were conducted in parallel, and the result showed that positive association was evident only in TDT analysis. Similarly, previous studies^{12,13} also found no correlation between GABRB3 and ASD. The present finding, therefore, suggests that GABRB3 gene may be not associated with ASD in the general population where the possibility of

crossovers is higher than that in a family group. An isolated group with less frequent inflow of population may be more useful for genetic association studies, since the linkage between the etiological gene and other alleles in the vicinity is more consistent.¹⁸ In ASD, a rare disorder, the frequency of casual genes of ASD would also be low. A considerable number of study subjects have to be recruited to obtain sufficient gene frequency for statistical power. Therefore, TDT analysis can be a more useful association study of disorders with rare prevalence, such as autism.¹⁹ Moreover, TDT can overcome the stratification bias that occurs due to different sample compositions from the general population, a crucial weak feature of case-control genetic association studies.²⁰

This study provides preliminary evidence that GABRB3 gene could be associated with ASD in Korea, and additionally supports the functional roles for genetic variants within the GABA receptor gene complex in Korean ASD. However, this study has the limitation of a relatively small sample size for population-based association study which may influence type II error. Furthermore, we did not apply the objective diagnostic tools for ASD such as the Autism Diagnostic Interview and the Autism Diagnostic Observation Schedule on healthy comparison subjects as well as children with ASD. Further studies with larger sample size and application of objective diagnostic tools are required to confirm our findings.

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