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No Association Study of SLC6A4 Polymorphisms with Korean Autism Spectrum Disorder*

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한국인 자폐스펙트럼장애와 SLC6A4 유전다형성의 연관 연구*

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ABSTRACT

Objectives : The serotonin transporter gene *(SLC6A4)* is one of the most widely studied candidate genes in autism spectrum disorder(ASD), but there have been conflicting results from studies into the association between *SLC6A4* and ASD. The aim of this study was to evaluate the association between single nucleotide polymorphisms(SNPs) in the *SLC6A4* gene and ASD in the Korean population.

Methods : We selected 12 SNPs in *SLC6A4* and observed the genotype of 151 Korean ASD trios. We tested the family-based association for each individual polymorphism and haplotype by using the standard TDT method in Haploview(http://www.broad.mit.edu/mpg/haploview/).

Results : Through transmission-disequilibrium testing and haplotype analysis, we could not find any statistically significant transmitted allele or haplotype. In addition, a case-control association test with Korean HapMap data did not reveal any statistical significance.

Conclusion : Although serotonin-related genes must be considered candidate genes for ASD, we suggest that common SNPs of *SLC6A4* are not important markers for associations with Korean ASD.

KEY WORDS : Autistic disorders · Serotonin transporter · Genetic polymorphism · Haplotypes · Association.

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Introduction

Autism spectrum disorders (ASDs) are neuropsychiatric developmental disorders characterized by impairment in social communication and a preference for repetitive and solitary interests and behaviors. Several family and twin studies have reported the role of genetics in the etiology of autism.[1] In association genetic studies for ASDs, several neurotransmitter genes such as serotonin-related genes are considered as candidate genes because of the possible involvement of neurochemical effects in pathogenesis of ASDs.

Serotonin (5-hydroxytryptamine or 5-HT) has been considered to play an important role in ASD development. A relatively high platelet serotonin level was observed in a subset of ASD subjects and their first-degree relatives[2]; the blood serotonin level was also found to correlate with the verbal intelligence quotient (IQ) and the severity of the disorder.[3-5] The use of selective serotonin reuptake inhibitor drugs (SSRIs) was found to improve some symptoms such as repetitive behavior, aggression, and language use in individuals with autistic disorder.[6-8] Numerous genes, for example, the serotonin transporter gene, the tryptophan hydroxylase gene, and the monoamine oxidase A gene, are involved in regulating serotonin levels, and some of them have been screened in ASD individuals.[9-12]

The serotonin transporter is a major component of the serotonergic system and is thought to play a role in autism pathogenesis. The serotonin transporter gene (*SLC6A4*) is one of the most widely studied candidate genes for ASD; it is located in chromosome 17q11.2 and has 14 exons. Several linkage studies and genome-wide screening studies have found evidence of a linkage near the serotonin transporter gene.[13-15] Some studies have also reported an association between autism and polymorphic markers in the *SLC6A4* gene, such as 5-HTTLPR insertion-deletion polymorphisms in the promoter region. The association between the 5-HTTLPR polymorphism of *SLC6A4* and ASD has produced controversial results that touch upon ethnic diversity, methods of genetic analysis, and symptom profiles of ASD.[9)14)16-20] It is suggested that hyperserotonemia in ASD may be associated with the effects of regulation and expression of *SLC6A4* via the 5-HTTLPR promoter polymorphism.[21)22] The results of the association analysis using single nucleotide polymorphisms (SNPs) of *SLC6A4* (i.e., rs2020942, rs2066713, and rs2020936) in several studies were controversial. Kim et al.[17] reported an association between autism and these SNPs in 115 trios examined, but their results were not consistent with those of McCauley et al.[14] or Wu et al.[23] In their extensive study with large cohort samples, Ramoz et al.[24] reported the absence of a linkage or association with 9 SNPs and 5-HTTLPR covering *SLC6A4* or any haplotypes.

By using the transmission/disequilibrium test (TDT), we found a significant preferential transmission of the long allele of 5-HTTLPR in Korean ASD.[25] To further investigate the role of *SLC6A4* in susceptibility of Korean population to ASD, we evaluated the association between ASD and common SNPs of *SLC6A4* by using 151 ASD trios in the Korean population.

Methods

1. Subjects

The subjects were recruited from a family-based genetic association study of ASD conducted by the same research group.[25)26] Subject ascertainment and diagnostic methods have been described previously. Briefly, ASD was diagnosed using the Autism Diagnostic Interview-Revised (ADI-R)[27] and the Korean version of the Autism Diagnostic Observation Schedule (ADOS)[28] with the best estimates of 2 board-certified child psychiatrists. Subjects diagnosed to have or strongly suspected of having neurofibromatosis, tuberous sclerosis, metabolic encephalopathies, Down's syndrome, Fragile X syndrome, or other known chromosomal abnormalities were excluded. We obtained written informed consent from all participants, and the study was approved by the Institutional Review Boards (IRB) of the participating institutions. This study included 151 complete trios, consisting of patients with ASD (79.9±35.6 months, 86.1% males,

87.4% autism, 13.5% Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS), and 1.6% Asperger's disorder) and their biological parents. Their psychological characteristics were almost similar, as described previously.[26]

For a case-control association study, we used Korean HapMap project data of 7 SNPs (rs3813034, rs1042173, rs6352, rs140701, rs2020942, rs6354, and rs2020936) in the *SLC6A4* gene from 90 Korean adult samples with a 1 : 1 male to female ratio (http://sysbio.kribb.re.kr:8080/khapmap/index.jsp).

2. Genotyping

Blood samples from all subjects were collected in tubes containing EDTA and stored at −70℃. Genomic DNA was extracted using the G-spin Genomic DNA Extraction Kit (Intron, Daejeon, Korea).

We evaluated the genetic structure of *SLC6A4* by using the Entrez SNP Database (http://www.ncbi.nlm.nih.gov/) and determined SNP in the coding region (cSNP) and a common genetic variation of *SLC6A4* (i.e., SNPs in the gene region with minor allele frequencies >5% in 2 Asian populations). We selected 5 cSNPs (rs6352, rs28914834, rs28914832, rs2228673, and rs28914828), 4 SNPs in the intronic regions (rs3794808, rs140701, rs2020942, and rs2020936), 1 SNP in the 5'-untranslated region (rs6354), 1 SNP in the 3'-untranslated region (rs1042173), and 1 SNP in the 3'-near gene region (rs3813034) of *SLC6A4*. Genotyping was performed using the GoldenGate™ assay (Illumina, San Diego, USA).

3. Statistical analysis

To evaluate the data quality and the presence of genotypic errors, we evaluated the Hardy-Weinberg equilibrium and Mendelian inheritance of the genotypes within the trios. We tested the family-based association for each individual polymorphism and haplotype by using the standard TDT method in Haploview (http://www.broad.mit.edu/mpg/haploview/). Statistical significance was defined as $p<0.05$.

To evaluate the power of the samples to detect an excessive transmission of alleles, we used the program PBAT.[29] We assume that autism prevalence to be K= 0.006, with a targeted significance level of 0.05. In terms of the frequency of the disease allele, we used observed frequency of the highly transmitted allele and we employed the additive model.

Tests to compare the alleles, genotypes and haplotype frequencies of cases with those of Korean HapMap controls were conducted using SNPAlyze 5.0.4 (Dynacom, Chiba, Japan).

Results

The genotypic distribution for all the SNPs did not deviate from that expected based on the Hardy-Weinberg equilibrium. Four cSNPs (rs28914834, rs28914832, rs2228673, and rs28914828) were monomorphic. Information on these SNPs is given in Table 1. In the TDT analysis, we did not find any statistically significant overtransmitted SNP alleles in the ASDs (Table 2). Estimation of the pairwise linkage disequ-

Table 1. Information of single nucleotide polymorphism of the *SLC6A4* gene

Marker	Distance	Contig position	Location	Major allele	Frequency			
					Korean ASD patients	Korean	Chinese	Japanese
rs3813034		3261797	3' near gene	C	0.81	0.82	0.81	0.84
rs1042173	207	3262004	Exon 15	C	0.81	0.82	0.81	0.83
rs6352	5389	3267186	Exon 14	A	0.95	0.93	0.93	0.92
rs3794808	6989	3268786	Intron 13	A	0.81		0.81	0.84
rs140701	13728	3275525	Intron 9	A	0.81	0.83	0.79	0.83
rs2020942	22110	3283907	Intron 3	G	0.92	0.94	0.90	0.94
rs6354	25094	3286891	Exon 2	A	0.90	0.89	0.86	0.88
rs2020936	26010	3287807	Intron 1	A	0.90	0.88	0.86	0.88

Frequency of Korean population for each marker is obtained from the information by Korean HapMap database. And that of the other populations are obtained from the information by International HapMap database

Table 2. Transmission disequilibrium test of the *SLC6A4* gene in Korean autism spectrum disorders trios

SNP	Overtransmitted allele	T : NT	Ratio	Chi- square	p-value(df=1)
rs3813034	−	46 : 46	1.00	0.00	1.00
rs1042173	C	48 : 46	1.04	0.04	0.84
rs6352	A	13 : 11	1.18	0.17	0.68
rs3794808	A	47 : 46	1.02	0.01	0.92
rs140701	−	45 : 45	1.00	0.00	1.00
rs2020942	−	20 : 20	1.00	0.00	1.00
rs6354	A	32 : 23	1.39	1.47	0.22
rs2020936	A	31 : 23	1.35	1.19	0.28
Haplotype	Frequency	T : NT	Chi-Square		p-value(df=1)
CCAAGAA	0.80	48.0 : 42.0	0.40		0.53
AAGGGCG	0.11	24.0 : 30.0	0.67		0.41
AAGGAAA	0.07	20.0 : 16.0	0.44		0.51

Haplotypes were constructed with rs3813034+rs1042173+rs3794808+rs140701+rs2020942+rs6354+rs2020936. T : transmitted, NT : not transmitted

Table 3. Linkage disequilibrium matrix between SNPs of the *SLC6A4* gene in Korean autism spectrum disorder family data

D'/r^2	rs3813034	rs1042173	rs6352	rs3794808	rs140701	rs2020942	rs6354
rs1042173	1.00/0.98						
rs6352	1.00/0.01	1.00/0.01					
rs3794808	0.99/0.97	1.00/0.99	1.00/0.01				
rs140701	0.99/0.98	0.99/0.96	1.00/0.01	0.99/0.97			
rs2020942	0.89/0.28	0.95/0.31	1.00/0.01	0.95/0.31	0.89/0.28		
rs6354	0.95/0.51	0.95/0.50	1.00/0.01	0.95/0.50	0.95/0.51	1.00/0.01	
rs2020936	0.98/0.53	0.98/0.52	1.00/0.01	0.98/0.53	0.98/0.53	1.00/0.01	1.00/0.97

ilibrium(LD) to determine the extent of LD for the SNPs showed that all the SNPs were in strong disequilibrium(D'>0.9) with each other(Table 3). Because of strong LDs among the SNP markers, in the haplotype analysis, we found only 3 haplotypes consisting of 7 SNPs in *SLC6A4*, and we did not observe any significant association between the haplotypes and ASD(Table 2).

We used an odds ratio(OR) of 1.7 for carrying one disease allele. Considering the prevalence of ASD in 0.6% of general population, the power of TDT for rs 1042173, rs6352, rs3794808, rs6354, and rs2020936 was 0.373, 0.131, 0.378, 0.218, and 0.218, respectively. A type I error rate of 0.05 was used in all calculations.

In the case-control association analysis, we did not observe any statistically significant differences in allele, genotype, and haplotype frequencies between our ASD cases and the Korean HapMap controls(Table 4).

Discussion

Several previous reports have indicated the possibility of a linkage and/or association between *SLC6A4* and ASD. Kim et al.[17] revealed an association between autism and SNPs of *SLC6A4* in 115 trios. However, studies involving TDTs have failed to reveal any consistent evidence of this association. In the present study, we did not observe any statistically significant transmission from biological parents to Korean ASD children with more SNPs(i.e., 12 SNPs in the SLC 6A4 gene, of which 8 SNPs were analyzed with statistical tools) in the TDT and haplotype analysis. Moreover, the power values of our study were very small, and our study has potential limitations with regard to

Table 4. Case-control association test between the *SLC6A4* gene and autism spectrum disorder in Korean population

SNP		Dominant	Recessive	Codominant	Allele
rs3813034	Chi-square	1.14(df=1)	0.53(df=1)	2.05(df=2)	0.13(df=1)
	p-value	0.29	0.47	0.36	0.72
rs1042173	Chi-square	1.14(df=1)	0.53(df=1)	2.05(df=2)	0.13(df=1)
	p-value	0.29	0.47	0.36	0.72
rs6352	Chi-square	1.18(df=1)	1.96(df=1)	4.3(df=2)	1.00(df=1)
	p-value	0.28	0.16	0.12	0.32
rs140701	Chi-square	1.2(df=1)	0.93(df=1)	2.66(df=2)	0.31(df=1)
	p-value	0.27	0.33	0.26	0.58
rs2020942	Chi-square	0.8(df=1)	0.8(df=1)		0.73(df=1)
	p-value	0.37	0.37		0.39
rs6354	Chi-square	3.41(df=1)	0.01(df=1)	3.49(df=2)	0.19(df=1)
	p-value	0.07	0.96	0.17	0.66
rs2020936	Chi-square	3.41(df=1)	0.07(df=1)	3.41(df=2)	0.39(df=1)
	p-value	0.07	0.80	0.18	0.53

Haplotype	Frequency		Chi-square (df=1)	Permutation p-value	Global p-value (df=10)
	Case	Control			
C-C-A-A-G-A-A	0.76	0.75	0.19	0.65	
A-A-A-G-G-C-G	0.09	0.10	0.02	0.90	0.32
A-A-A-G-A-A-A	0.08	0.06	0.77	0.40	(Chi-square=11.51)
Others	0.06	0.10			

Haplotypes were constructed with rs3813034+rs1042173+rs6352+rs140701+rs2020942+rs6354+rs2020936

detection of causative variants or variants with more modest effects. Furthermore, the control subjects were not age and sex matched with the study subjects, and psychiatric disorders in the control subjects were not evaluated. Nonetheless, the results of the case-control analysis did not reveal any statistically significant results.

The reason for these differences in association results might be as follows. First, ethnic differences might contribute to different genetic associations. This study included only Asian Korean population, similar to the study of Wu et al.(175 Han Chinese trios)[23] ; thus, the sample population differed from that used in the study by Kim et al.(94 Caucasians, 7 Americans, 8 Asian Americans, and 6 Hispanics).[17] Second, the sample size of the study by Kim et al. was relatively small compared to that of our study. Therefore, the results of the study by Kim et al need to be considered. Finally, the genetic heterogeneity of ASDs and the possibility of involvement of other genes or environmental factors such as gene-gene interaction or gene-environmental interaction must be considered.

Although our study had certain limitations such as relatively small sample size and a lack of information on phenotypes and environmental risk factors, our findings suggest that common SNPs(minor allele frequency of more than 0.01) and haplotypes for *SLC6A4* do not appear to significantly contribute to ASD in the Korean population. Nonetheless, we believe that the association study of the 5-HTTLPR(not SNPs) marker for *SLC6A4* must be replicated in a larger Korean population, together with a phenotypic analysis, because of its functional effects and a previous report on positive association.[25]

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