Polymorphism in the Promoter Region of *SEMA5A* Is Associated with Sociality Traits in Korean Subjects with Autism Spectrum Disorders

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In this study, we evaluated the association between autism spectrum disorders (ASDs) and 10 single-nucleotide polymorphisms (SNPs) in the 5' region of the semaphorin 5A gene (*SEMA5A*) for 250 Korean trios including children with ASDs. Family-based association testing and haplotype analysis revealed a statistically significant association between rs194085 and multiple sociality traits with Korean ASDs in the dominant model (p<0.001, corrected p=0.035). This indicates that genetic variations in the 5' region of *SEMA5A* play a role in the genetic predisposition to sociality traits in Korean ASDs. **Psychiatry Investig 2017;14(6):876-878**

Key Words   Autism spectrum disorder, Semaphorin 5A gene, Polymorphism, Sociality.

INTRODUCTION

Genetic factors are thought to play important roles in the pathogenesis of autism spectrum disorders (ASDs), and several genome-wide association studies (GWAS) have evaluated large family sizes for linkage and association mapping. Weiss et al.[1] suggested that the semaphorin 5A gene (*SEMA5A*) is an autism susceptibility gene based on the results of a GWAS of 1031 multiplex autism families.

A previous report also observed down-regulation of *SEMA5A* from Epstein-Barr virus-transformed B lymphocytes from idiopathic autism patients.[2] In addition, *SEMA5A* expression was significantly lower in autistic brains than in control brains after adjusting for age at brain acquisition, post-mortem interval,

and sex.[1] In this study, we investigated whether *SEMA5A* is genetically associated with Korean ASDs.

METHODS

A total of 250 Korean ASDs family samples from the Korean Autism Genetic Consortium were used (including 173 multiplex families and 959 individuals) in this study as previous described.[3] To confirm ASD diagnoses, subjects were administered the Korean versions of the Autism Diagnostic Observation Schedule[4,5] and the Autism Diagnostic Interview-Revised (ADI-R).[6,7] The ADI-R is designed to elicit a full range of information needed to diagnose autism and assist in assessing ASD. The components of the diagnostic algorithm of ADI-R are subdivided into 3 main domains: qualitative abnormalities in reciprocal social interaction (designated "A"), qualitative abnormalities in communication ("B"), and repetitive and restricted behaviors and interests ("C"). The Asperger Syndrome Diagnostic Scale (ASDS) is designed to identify Asperger syndrome in children aged 5−18 years and contains 50 items grouped into 5 subgroups including sociality-related subscales.[8] The social responsiveness scale (SRS) is a 65-item rating scale

that generates scaled scores for specific symptom domains (social awareness, social cognition, social communication, social motivation, and autistic mannerisms), as well as a total score, which serves as an index of the severity of social deficits in ASD.[9] In order to test family-based associations for ASD quantitative traits for sociality, we selected 3 traits with quantitative clinical scores using tools such as the total score of subdomain A for a diagnostic algorithm in the Korean version of ADI-R, total value of the subscale for sociality in ASDS, and converted total score for SRS (Supplementary Table 1 in the online-only Data Supplement).

The study protocol was approved by the Institutional Review Board of Eulji University (IRB No. EU 11-13). Informed consent was confirmed (or waived) by the Institutional Review Board and written informed consent was obtained from the parents of all subjects.

Single-nucleotide polymorphisms (SNPs) were selected as common tagSNPs suggested by International HapMap project website (minor allele frequencies>0.1, $r^2=0.8$ in Chinese and Japanese populations) in a 100-kb region from rs10513025, which has been reported as a marker for SNPs in a previous GWAS study.[1] Nine SNPs (rs218052, rs194085, rs150630, rs390, rs160499, rs385, rs41457, rs395, and rs41463) and the rs10513025 SNP were examined in this study. The SNPs were genotyped in genomic DNA from blood using the GoldenGate™ Assay (Illumina, San Diego, CA, USA).

Family-based association tests for each SNP and haplotype were assessed using the family-based Association Test (FBAT) program package (http://www.biostat.harvard.edu/˜fbat/default.html). To analyze the multiple correlated traits for sociality simultaneously, we apply FBAT-GEE method.

This multivariate approach can substantially reduce the power loss associated with multiple testing approaches.[10] To handle multiple comparison problems, statistical significance was specified by means of Bonferroni correction.

RESULTS

SNP rs10513025, which was reported to be associated with ASDs in a previous GWAS study, was monomorphic in this study sample. In the linkage disequilibrium (LD) test for each pair of markers, an LD block containing 3 SNPs (rs218052, rs194085, and rs150630) was observed with relatively strong LD values (0.57<D'<0.94) (Supplementary Figure 1 in the online-only Data Supplement). There was no significant difference in SNP markers for any model and any mode in FBAT for discretion affection status (Supplementary Table 2 in the online-only Data Supplement). Power was lacking to detect an association with disease affection (Supplementary Table 3 in the online-only Data Supplement). In haplotype analyses of 3 SNPs

in the LD block, one haplotype showed a statistically significant association with both the additive (Z=-2.185, p=0.029) and dominant (Z=-2.041, p=0.041) models (Supplementary Table 4 in the online-only Data Supplement). However, the significance disappeared after multiple testing correction. The results of FBAT with specific traits for sociality revealed a significant difference in rs194085 with a quantitative trait for SRS-summary score in the dominant and additive models (dominant model C allele Z=3.170, p=0.001, corrected p=0.020; additive model C allele Z=2.242, p=0.025). In addition, we observed a genetic association with rs390 for the SRS score (dominant model C allele Z=2.216, p=0.027). Furthermore, when we applied the FBAT for 3 traits with additional quantitative data for sociality, the results revealed stronger associations at each SNP. Particularly, statistical significance for the genetic association with rs194085 in the dominant model remained after multiple testing correction (biallelic mode C allele Z=3.170, p<0.001, corrected p=0.005) (Table 1). rs194085 was also statistically significant in the multiallelic model (df=2, $\chi^2=21.85$, p=0.001, corrected p=0.020).

DISCUSSION

As a hypothesis-free approach, GWAS examining a sufficiently large sample and replicating the most significant SNPs in an independent sample with sufficient power is thought to avoid false-positive findings. However, GWAS cannot typically replicate previous findings. In this study, we found no association between polymorphisms in the 5' region of *SEMA5A* with Korean ASDs. This may be because of a lack of power, heterogeneity across populations, technical bias, population stratification, or environmental effects.[11] Anney et al.[12] also failed to replicate the findings for this 5' region of *SEMA5A* in 1558 autism families, likely because of a lack of power. Moreover, in contrast to the "common disease-common variant" genetic model underlying GWAS, risk alleles with low minor allele frequency or rare variants may be involved in causing ASDs. Recently, a role for rare causal variants and *de novo* mutations emerged in the genetic architecture of ASDs.[13] In this study, common SNPs were selected, but not all polymorphisms could be examined. Ethnic differences may have affected the results because SNP rs10513025 showed a positive association with ASDs in GWAS and is monomorphic in the Korean population.[1]

In this study, we focused on sociality-related traits in Korean ASDs and found statistically significant associations between rs194085 SNP and sociality traits. The alleles of rs194085 showed different affinities to several transcription factors at rSNPs MAPPER2 (http://genome.ufl.edu/mapper/mapperrun?init=rs), such as the TATA box binding protein, which is

Table 1. The family-based association test results for sociality related multiple traits of Korean ASD with single nucleotide polymorphisms in SEMA5A gene using bi-allelic mode

Model	SNPs	Allele	Frequency	SRS			Multiple traits for sociality		
				N	Z	p	N	χ^2	p
Additive	rs218052	G	0.328	104	0.936	0.349	100	0.597	0.897
	rs194085	C	0.238	76	2.242	0.025	75	12.258	0.007
	rs150630	A	0.384	102	0.147	0.883	97	0.486	0.922
	rs390	C	0.473	113	1.847	0.065	108	5.896	0.117
	rs160499	T	0.312	95	-0.205	0.838	92	0.154	0.985
	rs385	C	0.358	104	1.329	0.184	98	2.917	0.405
	rs41457	G	0.149	70	1.547	0.122	66	4.844	0.184
	rs395	G	0.391	110	-1.334	0.182	106	6.383	0.094
	rs41463	G	0.116	49	-0.568	0.570	47	2.617	0.454
Dominant	rs218052	G	0.328	88	1.222	0.222	86	1.808	0.613
	rs194085	C	0.238	73	3.177	0.001	72	20.338	<0.001
	rs150630	A	0.384	75	0.018	0.985	73	1.15	0.765
	rs390	C	0.473	80	2.216	0.027	77	10.99	0.012
	rs160499	T	0.312	84	-0.519	0.604	81	1.719	0.633
	rs385	C	0.358	87	0.958	0.338	82	1.413	0.703
	rs41457	G	0.149	69	1.664	0.096	65	3.595	0.309
	rs395	G	0.391	86	-0.928	0.354	83	6.558	0.087
	rs41463	G	0.116	48	-0.217	0.828	46	2.798	0.424

ASD: autism spectrum disorder, SRS: Social Responsiveness Scale, SNPs: single-nucleotide polymorphisms, N: number of family, Z: Z-score

thought to function as a key component of the general transcription machinery and plays a role in embryonic and nervous system development.[14,15] In conclusion, this study suggests that rs194085, an SNP that may affect the expression of genes involved in neurogenesis, is associated with sociality-related Korean autistic phenotypes and possibly plays a role in the pathogenesis of ASDs.

Supplementary Materials

The online-only Data Supplement is available with this article at https://doi.org/10.4306/pi.2017.14.6.876.

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