Neuroscience Letters 479 (2010) 197–200

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Association study between single nucleotide polymorphisms in promoter region of *AVPR1A* and Korean autism spectrum disorders

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ARTICLE INFO

Article history:
Received 20 February 2010
Received in revised form 22 April 2010
Accepted 13 May 2010

Keywords:
Autism spectrum disorders (ASD)
Single nucleotide polymorphisms (SNPs)
Arginine vasopressin receptor 1A (AVPR1A)
Family-base association study

ABSTRACT

To determine the association between arginine vasopressin receptor 1A gene (*AVPR1A*) and autism spectrum disorders (ASDs), we examined 3 single nucleotide polymorphisms (SNPs), namely, rs7294536, rs3759292, and rs10877969, in the promoter region of *AVPR1A* by using a family-based association test (FBAT) in 151 Korean trios. Our results demonstrated a statistically significant association between autism and SNPs (additive model: rs7294536, $\chi^2 = 9.328$, $df = 2$, $P = 0.002$; rs10877969, $\chi^2 = 11.529$, $df = 2$, $P < 0.001$) as well as between autism and haplotype analysis (additive model: $\chi^2 = 14.122$, $df = 3$, $P = 0.003$). In addition, we found that ADI-R scores calculated by using a diagnostic algorithm for *failure to develop peer relationships* (A2) were higher in subjects having the AA genotype than in subjects having the AG and GG genotypes of rs7294536. Thus, our study provides evidence for a possible association between these SNPs and the phenotype of ASDs.

Autism spectrum disorders (ASDs) encompass a broad range of neurodevelopmental deficits, including stereotypic behaviors and marked impairment in communication and social functioning [1]. Recent reports have estimated the prevalence of autism to be approximately 157 in 10,000 and have estimated the prevalence of ASDs to be much higher [4,19]. Etiology studies on numerous twins and families suggest a strong genetic component associated with ASD [2,9]. Genes related to neuropeptides, neurotransmitters, neurotrophins, synaptic plasticity, and neuroimmunity have been suggested to play a role in the pathogenesis of ASDs, and many different neural developmental processes and molecular pathways have been studied [20]. Although strong evidence exists for the heritability of ASDs, their exact neuropathophysiology remains obscured because of locus heterogeneity and clinical variability. Genome-wide association studies and linkage analysis in multiplex ASD families have suggested several promising chromosomal regions. An extended family study of autism reported a linkage peak on chromosome 12q14.2-14.3 containing the arginine vasopressin receptor A gene (*AVPR1A*) [18].

AVPR1A, a G-protein-coupled receptor activated by the binding of arginine vasopressin, stimulates phospholipase C [5], and induces the release of Ca^{2+} from the endoplasmic reticulum. It may be involved in neuronal development, memory formation, synaptic plasticity and neuronal survival via protein kinase C activation, and calmodulin MAP kinase and MAPK/ERK activation [6,23–25,33]. An early report using V1a knockout mice showed that vasopressin receptor 1A may be involved in the regulation of social interaction and may be associated with social behavioral deficits such as autism and schizophrenia [8]. The *AVPR1A* 5′ flanking region has been investigated in a series of studies across diverse species, from voles to primates and humans, which have repeat sequences in the *AVPR1A* 5′ flanking region. It is known that variable repeat sequences in the *AVPR1A* 5′ flanking region are associated with differences in social behaviors within and between species. Behaviors varying in accordance with *AVPR1A* expression are well-documented. For example, some prairie voles

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doi:10.1016/j.neulet.2010.05.050

have a long microsatellite allele in the *AVPR1A* promoter region and show higher expression levels of *AVPR1A* than other voles. The same voles demonstrate more social interaction than the counterparts of the same species possessing a short allele [10]. In humans, *AVPR1A* expression has been studied for its potential association with social behaviors and psychiatric disorders [3]. Expression levels of AVPR1A may be altered across brain regions in a cell-type dependent manner rather than in a globally regulated manner [13]. Hammock and Young suggested that individual genetic differences in AVPR1A expression patterns may contribute to variation in sociobehavior traits [11].

Several studies have reported that the microsatellite polymorphisms of *AVPR1A* are associated with social activity, such as altruism [16], age at first intercourse [22], human pair-bonding behavior [26], and prepulse inhibition [17]. They suggested that polymorphisms of these repeats affect human behaviors and psychiatric symptoms. Although other genetic and environmental causes may exist for ASDs, microsatellite polymorphisms in the 5′ flanking region of *AVPR1A* have been suggested as strong candidate genetic markers for traits related to social interaction. The results of previous association studies between *AVPR1A* polymorphisms involving ASDs families have been controversial. Kim et al. first studied the polymorphism complex $(CT)_4$-TT-$(CT)_8$-$(GT)_{24}$ repeats (RS1) and $(GATA)_{14}$ tetranucleotide repeats (RS3) in *AVPR1A*, and found nominally significant transmission disequilibrium of 332 bp in RS3 repeats in 115 ethnically unmatched ASD families consisting of Caucasian (94), African-American (7), Asian-American (8), and Hispanic (6) families [15]. Wassink et al. replicated the results of the association test between microsatellites (RS3 and RS1) and autism, studying 65 Caucasian families [27]. Our previous study also showed that the association of the microsatellites (RS3, RS1) in *AVPR1A* with 151 ASD families within the Korean population was statistically significant [28]. However, Yirmiya et al., who studied 114 Israeli families, found a significant association between a new marker, AVR, which is located on the intron of *AVPR1A*, and ASDs. They reported that the measures of ADI-R associated haplotypes of the microsatellite markers in the 5′ flanking region had statistically significant *p*-values [29].

Single nucleotide polymorphisms (SNPs) are a common type of polymorphism involving a small number of alleles than microsatellites. Although many studies have explored the relationship between microsatellites and autism or human behavior, association studies on SNPs in *AVPR1A* and psychiatric disorders have been seldom conducted. The present study investigates their association with Korean ASD families using extended SNPs that may regulate *AVPR1A* transcriptional level in a core regulatory region. This study also attempts to establish a genotype–phenotype correlation between SNPs and Korean ASD families.

Subjects were recruited from a family-based genetic association study of ASD that we conducted. Subject ascertainment and diagnostic methods have previously been described [7,14,32,33]. Briefly, the ASD probands were diagnosed using the Autism Diagnostic Interview-Revised (ADI-R) [30] and the Korean version of the Autism Diagnostic Observation Schedule (ADOS) [31], together with the best estimates of 2 board-certified child psychiatrists. Subjects diagnosed with or strongly suspected of having neurofibromatosis, tuberous sclerosis, any kind of metabolic encephalopathy, and known chromosomal abnormalities were excluded from the study. None were diagnosed with clinically significant partial seizure disorder. The present study included 151 complete trios, comprising patients with ASD (79.9 ± 35.6 months, 86.1% male, 87.4% with autism, 13.5% with PDD-NOS, and 1.6% with Asperger's disorder) and their biological parents. The psychological properties of study were fairly similar, as described previously [14].

Blood samples from 151 trios were collected in EDTA tubes and kept at $-70\,^{\circ}C$. Genomic DNA was prepared using the G-spin Genomic DNA Extraction Kit (Intron, Daejeon, Korea). Gene structure of *AVPR1A* was 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 (www.hapmap.org). A distance of 5 kb from the 5′ flanking region of *AVPR1A* was set as the study region. Common SNPs, with minor allele frequencies (MAFs) of >20% in 2 Asian populations (Chinese and Japanese), were assessed. Three SNPs (rs10877969, rs3759292, and rs7294536) located in the promoter region were selected for study and genotyped using the GoldenGate™ Assay (Illumina, San Diego, CA, USA). After the chip-based genotyping assay, the 3 selected markers were reassessed by sequencing.

We used the sum of 3 behavioral domain scores of the ADI-R diagnostic algorithms as a measure of severity of the following symptoms: qualitative abnormalities in reciprocal social interaction, qualitative abnormalities in communication, and restricted, repetitive, and stereotyped patterns of behavior. The ADI-R item scores range from 0 (no abnormality) to 3 (most abnormal), and a score of 3 should be converted to 2 for the algorithm score. In addition, 8 (not applicable) or 9 (unknown or not asked) was converted to 0, as specified in the algorithm.

Mendelian inheritance error for each individual polymorphism was checked by PedCheck (v.1.1). Allele frequency, Hardy–Weinberg equilibrium, and linkage disequilibrium (LD) D' values between each pair of SNPs were evaluated by the transmission disequilibrium test (TDT) method in Haploview 3.2 (http://www.broad.mit.edu/mpg/haploview).

Family-based association tests for each individual polymorphism and haplotype were assessed with the TDT using the Family-based Association Test (FBAT) program package (http://www.biostat.harvard.edu/˜fbat/default.html) [12]. FBAT defines an informative family as "Having at least one parent contributing to the variance of the offspring genotype during transmission." This is based on the assumption that transmission follows Mendelian rules. This means that for most models, an informative family occurs if you have at least 1 heterozygous parent. Having 2 homozygous parents gives you exactly what the offspring genotype will be. FBAT tests rely on the comparison between expected transmission and observed transmission of alleles. With 2 homozygous parents there is nothing to compare and the family is uninformative. When using FBAT, the number of informative families depends on the genotypic information and genetic model. Transmitted alleles from heterozygous parents were extracted, and used to compute $(^2$ and P values with the aid of available modes and models. HBAT, the haplotype version of the FBAT program, was used to find haplotypes with a >5% frequency of association with ASDs. Haplotype tests were performed using permutation ($N = 100{,}000$ cycles) by the HBAT Monte Carlo option. Furthermore, the D' value between the SNPs was estimated by the FBAT program package. Analysis of variance (ANOVA) was used to compare the ADI-R domain scores according to the specific genotype of each SNP. SPSS (ver. 15.0) was used for the analysis. Statistical significance was defined as $P < 0.05$.

Of the 151 data sets, Mendelian inheritance errors were found in 2 trios, and these 2 trios were excluded from the transmission test and haplotype analysis. In the LD test for pairs of markers, the 3 SNPs were tightly linked ($0.89 < D' < 0.99$) (Table 1). The transmission data, determined by FBAT, are shown in Table 1. With regard to the additive model in the bi-allelic mode, we found significant *P*-values for rs7294536 ($Z = 3.054$, $P = 0.002$) and rs10877969 ($Z = 3.395$, $P < 0.001$) in the FBAT. With regard to the dominant models in the bi-allelic mode, the transmission test showed that the G alleles of rs7294536 and rs10877969 have protective effects in patients with ASDs ($Z = -3.054$ with $P = 0.002$ and $Z = -3.054$ with $P < 0.001$, respectively). We confirmed these results in recessive models in the bi-allelic modes. The FBAT analysis results for