Family-based association study of the arsenite methyltransferase gene (AS3MT, rs11191454) in Korean children with attention-deficit hyperactivity disorder
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We examined the association between the selected polymorphisms in two candidate genes, the arsenite methyltransferase gene (AS3MT, rs11191454) and the inter-α-trypsin inhibitors heavy chain-3 gene (ITIH3, rs2535629), and attention-deficit hyperactivity disorder (ADHD) in a Korean population. A total of 238 patients with ADHD, along with both of their biological parents, were recruited. The children were administered intelligence quotient tests, whereas their parents completed the Child Behavior Checklist. In the transmission disequilibrium test on 181 trios, we found overtransmission of the A allele at the AS3MT rs11191454 polymorphism in children with ADHD (χ² = 8.81, P = 0.003). However, there was no preferential transmission at the ITIH3 rs2535629 polymorphism (χ² = 0.14, P = 0.707). Our results provide preliminary evidence for the overtransmission of the A allele at the AS3MT rs11191454 polymorphism in ADHD. Psychiatr Genet 25:26–30 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction
Attention-deficit hyperactivity disorder (ADHD) is a common disorder that has a 4–10% prevalence rate among elementary school children and is primarily characterized by inattention, impulsivity, and hyperactivity (Biederman and Faraone, 2005). The Diagnostic and Statistical Manual of Mental Disorders, 4th ed., classifies ADHD into three subtypes, inattentive, hyperactive/impulsive, and combined, on the basis of clinical phenomenology (American Psychiatric Association, 2000). ADHD of the inattentive type presents with symptoms including being easily distracted, being forgetful, daydreaming, being disorganized, having poor concentration, and having difficulty completing tasks. ADHD of the hyperactive/impulsive type presents with excessive fidgetiness and restlessness, talking a lot, hyperactivity, and difficulty waiting and remaining seated. ADHD of the combined type is a combination of the two other subtypes.

ADHD has an estimated heritability of ~80% and has been conceptualized as a complex, polygenic disorder (Faraone and Biederman, 1998). A large number of studies on the different candidate genes for ADHD have been published, most of which have focused on genes that are involved in the dopaminergic, noradrenergic, and serotonergic neurotransmission systems (Banaschewski et al., 2010; Akutagava-Martins et al., 2013). Candidate genes associated with increased risk for ADHD on the basis of pooled odds ratios across nine or more studies are the dopamine receptors D4 and D5 (DRD4 and DRD5), dopamine transporter 1 (DAT1), serotonin transporter (SLC6A4), and serotonin receptor 1B (HTR1B; Gizer et al., 2009; Akutagava-Martins et al., 2013). Other candidate genes, such as nicotinic acetylcholine receptor 4 (CHRNA4) and synaptosomal-associated protein 25 kDa (SNAP25), have been also associated with ADHD, although in a smaller number of studies (Gizer et al., 2009). Genes such as dopamine receptor D2 (DRD2), adrenergic-a2A receptor (ADRA2A), and noradrenaline transporter protein 1 (SLC6A2) have shown inconsistent results and did not reach significance in meta-analyses (Gizer et al., 2009); however, they remain as interesting candidate genes.

Recently, the Cross-Disorder Group of the Psychiatric Genomics Consortium (2013) analyzed genomewide single-nucleotide polymorphism (SNP) data for five major psychiatric disorders (ADHD, autism spectrum disorder, bipolar disorder, major depressive disorder, and schizophrenia) in European ancestry to identify risk loci with shared effects on the five disorders. This group found that SNPs at four loci surpassed the cutoff for genomewide significance (P < 5 × 10−8) in the primary analysis. The strongest association signal was on chromosome 3 at an intronic SNP within the inter-α-trypsin
inhibitors heavy chain-3 (ITIH3) gene (rs2535629). The second strongest signal was in an intron of the arsenite methyltransferase (AS3MT) gene on chromosome 10q24 (rs11191454). AS3MT, an enzyme that is encoded by the AS3MT gene in humans, catalyzes the transfer of a methyl group from S-adenosyl-L-methionine to trivalent arsenical and may play a role in arsenic metabolism (Lin et al., 2002). This group also found genomewide significant associations within two L-type voltage-gated calcium channel subunit genes, CACNA1C (rs1024582) and CACNB2 (rs2799573). These results suggest that variation in specific SNPs has pleiotropic effects on psychopathology.

Arsenic is a natural component of the earth’s crust and is widely distributed throughout the environment in air, water, and land. This element is highly toxic in its inorganic form. People are exposed to elevated levels of inorganic arsenic (IA) through drinking contaminated water, using contaminated water in food preparation and irrigation of food crops, industrial processes, eating contaminated food, and smoking tobacco (Smedley and Kinniburgh, 2002). Arsenic exposure is known to induce neurotoxicity. Neurotoxicological impairments induced by arsenic exposure include poor cognitive performance and disturbances in visual perception, psychomotor speed, attention, speech, and memory (Rosado et al., 2007). In general, humans can metabolize ingested IA using AS3MT. The metabolic action of AS3MT is considered to be associated with the accumulation profile of various arsenic compounds in the body and consequently with susceptibility to the toxic effects of arsenic.

In this study, we aimed to examine the association between two selected polymorphisms, which were identified as having shared effects on five major psychiatric disorders, and ADHD in a Korean population. On the basis of the finding that four SNPs underlie the shared genetic effects of the five disorders, we hypothesized that these specific variants might be associated with lower intellectual functioning, which is associated with a greater risk for both psychiatric disorders (Trivedi, 2006) and ADHD (Jepsen et al., 2009). Because the two SNPs within the L-type voltage-gated calcium channel subunits (rs1024582 and rs2799573) were known to be almost monomorphic in the Korean population (KoreanHapmapproject), we examined the remaining two SNPs, rs2535629 on chromosome 3 and rs11191454 on chromosome 10.

Methods
Participants
Patients with ADHD (6–15 years) and both of their biological parents were recruited from child psychiatric outpatient clinics at university hospitals in Korea. Among the 260 children with ADHD originally approached for the study, 238 (91.5%) participated in this study. To confirm the diagnosis of ADHD, the Korean Kiddie-Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version (SADS-PL; Kim et al., 2004) was administered to all participants and their parents. Kiddie-SADS-PL is a version of the SADS-PL that is designed for school-aged children between 6 and 18 years old; it is administered by interviewing the parent(s) and the child, and finally summary ratings are achieved, which include all sources of information (parent, child, school, chart, and other; Kaufman et al., 1997). Exclusion criteria were as follows: (a) a history of pervasive developmental disorder, mental retardation, bipolar disorder, psychotic disorder, obsessive–compulsive disorder, or Tourette’s syndrome; (b) a history of neurological disorder; and (c) the presence of learning disabilities or language disorders. We provided detailed information about the study and then obtained written informed consent from both parents and the children. The study protocol was approved by the institutional review board of the Seoul National University Hospital.

Measurements
The parents completed the Child Behavior Checklist. The Child Behavior Checklist is a parent-reported questionnaire in which the child is rated on various behavioral and emotional problems (Achenbach, 1991; Oh et al., 1997). We examined the attention problem and thought problem scores in this study. All data are presented as t-scores, which were adjusted for age and sex. Higher t-scores indicate fewer problems.

The children were administered the abbreviated form of the Korean Educational Development Institute’s Wechsler Intelligence Scales for Children (KEDI-WISC; Park et al., 1996), which consist of the following scales: vocabulary, arithmetic, picture arrangement, and block-design tests. The sum of the age-adjusted t-scores for the four subtests was used to estimate full-scale IQs. The correlation between the abbreviated version and full version of the KEDI-WISC for full-scale IQs was 0.98 (Kim and Kim, 1986). The parents, children, and examiner of the IQ test were blinded to the children’s genetic data.

Genotyping
Genomic DNA was extracted from blood (stored frozen) using the G-DEXTM II Genomic DNA Extraction Kit (Intron, Korea) according to the manufacturer’s protocol. The detection of SNPs was based on analysis of primer extension products generated from previously amplified genomic DNA using a chip-based MALDI-TOF mass spectrometry platform (Sequenom Inc., San Diego, California, USA). The general procedures were performed according to the manufacturer’s standard protocol. Oligonucleotide primers (5’-ACG TTG GAT GGA ACT GAG ATA CGG AAA TGC and 5’-ACG TTG GAT GGG ACT CAC TTT CCT GTT TGG for rs11191454, and 5’-ACG TTG GAT GCT CTA GGC
CAT CAG CTG TC and 5′-ACG TTG GAT GCT GGA TCT AGT GCC ATC ACC for rs2535629) were used to generate PCR products.

Statistical analysis
A family-based study was performed to assess the genetic associations using transmission disequilibrium test statistics. The associations between quantitative neuropsychological measurements and specific SNPs were tested using linear regression analyses, as implemented in PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/). The potential confounding effects including age and sex were treated as covariates. The ‘perm’ option was used to generate a single-point empirical P-value (EMPI) to correct for the non-normal trait distribution using the adaptive permutation approach. Moreover, we transformed the mean and SD of the quantitative measurements after log transformation to obtain a ‘standardized’ β (a regression coefficient), allowing β to be comparable across different phenotypes. The criterion for significance was set at EMPI less than 0.025 (= 0.05/2 SNPs). Hardy–Weinberg equilibrium and Mendelian errors were examined using the PLINK options ‘–hwe’ and ‘–me’. Data handling and management were performed using R packages under the LINUX environment.

Results
A total of 238 children with ADHD (mean age = 8.86±2.03 years, 196 boys and 42 girls, mean IQ = 106.8±14.1) participated in this study. Table 1 shows the characteristics of the study participants. The family-based analysis included 181 trios consisting of an affected patient and his or her biological father and mother. This subset did not differ from the original participant pool in age, sex, or clinical characteristics (data not shown). Transmission disequilibrium test analysis of the AS3MT rs11191454 polymorphism showed biased transmission of the A allele (χ²=8.81, P=0.003). This biased transmission of the A allele was statistically significantly associated with adaptive permutations (EMPI=0.002). However, there was no preferential transmission of the ITIH3 rs2535629 polymorphism (χ²=0.14, P=0.707; Table 2). Linear regression analyses showed a negative, but not statistically significant, association between the A allele of n11191454 and IQ (EMPI=0.011) in 238 patients with ADHD (Table 3).

Discussion
In this study, we found preliminary evidence for over-transmission of the A allele at the AS3MT rs11191454 polymorphism in Korean children with ADHD.

Various studies have reported the neurologic sequelae of acute and chronic arsenic exposure in adults and children (Calderón et al., 2001; Tsai et al., 2003; Wasserman et al., 2004; Von Ehrenstein et al., 2007). The underlying mechanism is not yet clear, but arsenic inhibits the synthesis and liberation of acetylcholine in brain slices and increases monoamine activity in the nervous system (Rosado et al., 2007). Decreased locomotor activity and oxidative stress reactions due to arsenic toxicity may also affect the central nervous system and impair intellectual functioning (Von Ehrenstein et al., 2007).

Humans can metabolize highly toxic IA to monomethylated arsenic (MMA) and then dimethylated arsenic (DMA) through AS3MT. Previous epidemiological studies have shown that people with high percentages of DMA in their urine can excrete more arsenic from their body because of a high arsenic methylation capacity (Concha et al., 1998). In other studies, high concentrations or percentages of MMA in the urine were reported in populations with arsenic-related diseases when compared with healthy individuals (Del Razo et al., 1997; Valenzuela et al., 2005; Tseng, 2007).

Meza et al. (2005) found that three nonexonic SNPs (AS3MT rs12767543, rs3740393, and rs11191453) were significantly associated with the presence of DMA/MMA in the urine of the children (7–11 years) who ingested arsenic by drinking contaminated water. For example, the DMA/MMA levels for AS3MT (rs11191453) GA + GG were higher than those for AS3MT 35587 (rs11191453) AA, suggesting a low methylation capacity from MMA to DMA for the AA genotype. Recently, Gomez-Rubio et al. (2010) found significant associations of 10 unexonic SNPs

Table 1 Characteristics of 238 children with attention-deficit hyperactivity disorder

<table>
<thead>
<tr>
<th>Sex (males/females) [n (%)]</th>
<th>196/42 (82.4/17.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age distribution [n (%)]</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>32 (13.6)</td>
</tr>
<tr>
<td>7</td>
<td>48 (20.4)</td>
</tr>
<tr>
<td>8</td>
<td>46 (19.3)</td>
</tr>
<tr>
<td>9</td>
<td>34 (14.3)</td>
</tr>
<tr>
<td>10</td>
<td>26 (10.9)</td>
</tr>
<tr>
<td>11</td>
<td>16 (6.7)</td>
</tr>
<tr>
<td>12</td>
<td>9 (3.8)</td>
</tr>
<tr>
<td>13</td>
<td>19 (8.0)</td>
</tr>
<tr>
<td>14</td>
<td>4 (1.7)</td>
</tr>
<tr>
<td>15</td>
<td>4 (1.7)</td>
</tr>
<tr>
<td>ADHD subtype [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>123 (51.7)</td>
</tr>
<tr>
<td>Inattentive</td>
<td>82 (34.5)</td>
</tr>
<tr>
<td>Hyperactive-impulsive</td>
<td>12 (5.0)</td>
</tr>
<tr>
<td>Not otherwise specified</td>
<td>21 (8.8)</td>
</tr>
<tr>
<td>Comorbid disorder [n (%)]</td>
<td></td>
</tr>
<tr>
<td>Oppositional defiant disorder</td>
<td>35 (14.7)</td>
</tr>
<tr>
<td>Conduct disorder</td>
<td>3 (1.3)</td>
</tr>
<tr>
<td>Depressive disorder</td>
<td>9 (3.8)</td>
</tr>
<tr>
<td>Anxiety disorder</td>
<td>18 (7.8)</td>
</tr>
<tr>
<td>Tic disorder</td>
<td>11 (4.6)</td>
</tr>
</tbody>
</table>

ADHD, attention-deficit hyperactivity disorder.

Table 2 Allelic transmission calculated using the transmission disequilibrium test for rs11191454 and rs2535629 polymorphisms (number of trios = 181)

<table>
<thead>
<tr>
<th>Markers</th>
<th>Minor allele</th>
<th>Major allele</th>
<th>T:NT</th>
<th>χ²/d.f</th>
<th>P-value</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11191454</td>
<td>G</td>
<td>A</td>
<td>52:87</td>
<td>8.81</td>
<td>0.003</td>
<td>0.60</td>
</tr>
<tr>
<td>rs2535629</td>
<td>T</td>
<td>C</td>
<td>91:14</td>
<td>0.707</td>
<td>1.06</td>
<td></td>
</tr>
</tbody>
</table>

NT, not-transmitted; RR, relative risk; T, transmitted.
of the \( AS3MT \) polymorphism, including rs11191453, with urinary DMA/MM levels, indicating strong linkage disequilibrium. Although the \( AS3MT \) rs11191454 polymorphism is an intronic polymorphism, previous studies have suggested that this allele may adversely affect the expression and/or function of \( AS3MT \), thereby resulting in increased vulnerability to arsenic-induced neurotoxicity.

Our finding of a significant association of the A allele at the \( AS3MT \) rs11191454 polymorphism with ADHD and a negative, but nonsignificant, association of the A allele with low intelligence may be related to this increased arsenic-induced neurotoxicity in children with this allele. However, it is not possible to estimate the proportion of ADHD cases that might be attributable to the arsenic pathway, because we did not measure in-vivo concentrations of IA and did not analyze gene–environment interactions for arsenic exposure and ADHD susceptibility. Moreover, there was no association between the \( AS3MT \) rs11191454 polymorphism and the severity of attention problems measured by parents, suggesting that IA has more general effects on cognitive function and neurodevelopment rather than effects on neuropsychological domains specific to ADHD such as inattention.

Several limitations may have influenced the findings of this study. First, the sample size of the present study was relatively small for a genotypic analysis; hence, the results cannot be applied to the general population and should be interpreted carefully. Second, the participants included all subtypes of ADHD, which might have contributed to clinical heterogeneity. Third, we did not measure in-vivo concentrations of IA. Therefore, it is not possible to determine whether the association between the genetic polymorphism and the children’s intelligence is different according to the level of IA in the children. Finally, we genotyped only one specific SNP of \( AS3MT \) and \( ITIH3 \), respectively, and this may have been insufficient to fully address the association between these genes and ADHD. The study results must be replicated in larger populations and using more genetic markers.

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**Conflicts of interest**

There are no conflicts of interest.

**References**


