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Relation of polymorphism of arsenic metabolism genes to arsenic methylation capacity and developmental delay in preschool children in Taiwan

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ABSTRACT

Inefficient arsenic methylation capacity has been associated with developmental delay in children. The present study was designed to explore whether polymorphisms and haplotypes of arsenic methyltransferase (*AS3MT*), glutathione-S-transferase omegas (*GSTOs*), and purine nucleoside phosphorylase (*PNP*) affect arsenic methylation capacity and developmental delay. A case-control study was conducted from August 2010 to March 2014. All participants were recruited from the Shin Kong Wu Ho-Su Memorial Teaching Hospital. In total, 179 children with developmental delay and 88 children without delay were recruited. Urinary arsenic species, including arsenite (As^{III}), arsenate (As^{V}), monomethylarsonic acid (MMA^{V}), and dimethylarsinic acid (DMA^{V}) were measured using a high-performance liquid chromatography-linked hydride generator and atomic absorption spectrometry. The polymorphisms of *AS3MT*, *GSTO*, and *PNP* were performed using the Sequenom MassARRAY platform with iPLEX Gold chemistry. Polymorphisms of *AS3MT* genes were found to affect susceptibility to developmental delay in children, but *GSTO* and *PNP* polymorphisms were not. Participants with *AS3MT* rs3740392 A/G + G/G genotype, compared with *AS3MT* rs3740392 A/A genotype, had a significantly lower secondary methylation index. This may result in an increased OR for developmental delay. Participants with the *AS3MT* high-risk haplotype had a significantly higher OR than those with *AS3MT* low-risk haplotypes [OR and 95% CI, 1.59 (1.08–2.34)]. This is the first study to show a joint dose-response effect of this *AS3MT* high-risk haplotype and inefficient arsenic methylation capacity on developmental delay. Our data provide evidence that *AS3MT* genes are related to developmental delay and may partially influence arsenic methylation capacity.

1. Introduction

A review paper showed that many epidemiological studies have documented developmental neurotoxic effects in children with long-term arsenic exposure from contaminated milk powder (Dakeishi et al., 2006). Subsequently, a study found that exposure to arsenic from drinking water was associated with reduced intellectual function in 301 6-year-old children in Araihazar, Bangladesh (Wasserman et al., 2007). A study of 591 Mexican schoolchildren living in an area contaminated with both arsenic and lead showed that arsenic contamination can affect children's cognitive development, as well as leading to disturbances in visual perception, psychomotor speed, attention, speech, and memory, independent of any effect of lead (Rosado et al., 2007). Another study reported that arsenic exposure indices from water, urinary arsenic levels, and toenail arsenic concentration were inversely associated with motor function scores among 303 children in Bangladesh (Parvez

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et al., 2011). Grandjean and Herz, in a recent review paper, showed that environmental chemicals such as lead, methylmercury, and arsenic each contributed to developmental neurotoxicity (Grandjean and Herz, 2015). Increased urine arsenic levels were associated with attention impairment in school children living in an industrialized area of southwestern Spain, even at urinary levels of arsenic considered safe (Rodriguez-Barranco et al., 2016). In addition, children living in two New Hampshire (USA) school districts who were exposed to water arsenic concentrations ≥ 5 μg/L showed significant reductions in full-scale intelligence quotient scores, resulting in losses of 5–6 points in most indices (Wasserman et al., 2014). However, a study in rural Matlab, Bangladesh assessed 1799 infants, and did not find any significant effect of arsenic exposure during pregnancy on infant development (Tofail et al., 2009). Another study did not find any association between prenatal exposure to arsenic and neuropsychological development in 385 four-year-old children (Forns et al., 2014). Therefore, the epidemiological evidence is not yet solid in regard to neurotoxic risks, and the developmental effects of arsenic exposure need further investigation.

After exposure, biotransformation of arsenic takes place in the body and consists of a series of successive redox reactions and methylation. Arsenate (As^V) is reduced to arsenite (As^{III}), and then methylated to monomethylarsonic acid (MMA^V). MMA^V is then reduced to methylarsonous acid (MMA^{III}), which is methylated to dimethylarsinic acid (DMA^V) (Thompson, 1993). In the past, this biotransformation process was thought to be the arsenic detoxification pathway, but trivalent methylated metabolites are considered to be more toxic than inorganic arsenic (Petrick et al., 2001). The relative proportion of urinary arsenic species is considered an index of an individual's arsenic methylation capacity. In general, the profile of urinary arsenic in humans is 10–30% inorganic arsenic, 10–20% MMA^V, and 60–80% DMA^V (Vahter and Concha, 2001). Our previous studies showed that changes in the arsenic excretion profile are associated with increased risk of skin cancer (Hsueh et al., 1997), urothelial carcinoma (Pu et al., 2007), and developmental delay (Hsieh et al., 2014).

Epidemiological data show differences in individual susceptibility and response to arsenic exposure among the same population. Different susceptibility can be due to different arsenic metabolism capacity. Glutathione-S-transferase Omegas (GSTOs) (Zakharyan et al., 2001) and purine nucleoside phosphorylase (PNP) (Radabaugh et al., 2002) have been identified as candidate enzymes to catalyze the reduction of pentavalent inorganic arsenic. Methylation of trivalent arsenic is catalyzed by arsenic (+3 oxidation state) methyltransferase (AS3MT) in a reaction that uses S-adenosylmethionine (SAM) as the methyl group donor (Li et al., 2005). Genetic polymorphisms may alter enzyme function and possibly change the urinary metabolite profile. The Cross-Disorder Group of the Psychiatric Genomics Consortium analyzed genome-wide single-nucleotide polymorphism (SNP) data for attention-deficit hyperactivity disorder (ADHD), autism spectrum disorder, bipolar disorder, major depressive disorder, and schizophrenia in individuals of European ancestry to identify arsenite methyltransferase (*AS3MT*) rs11191454, which may play a role in ADHD (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). Recently, a study also found overrepresentation of the A allele of the *AS3MT* rs11191454 polymorphism in children with ADHD (Park et al., 2015). These findings suggest polymorphism of arsenic methylation enzyme may be related to ADHD. However, the mechanisms underlying how arsenic methylation capacity affects developmental delay are still unclear. In this study, we aimed to evaluate the association between polymorphisms of *AS3MT*, *GSTOs*, and *PNP* genes, and developmental delay. In addition, we also explored the association between polymorphisms of *AS3MT*, *GSTOs*, and *PNP* genes and arsenic methylation capacity. Putting these results together, we sought to elucidate the joint effects of *AS3MT*, *GSTOs*, and *PNP* gene polymorphisms and arsenic methylation capacity on the risk of developmental delay.

2. Materials and methods

2.1. Study participants

All participants were recruited from the Shin Kong Wu Ho-Su Memorial Teaching Hospital, a medical center located in northern Taiwan, between August 2010 and March 2014. Children with suspected developmental delays were referred to the medical center from local kindergartens, hospitals, and community centers near the hospital. All participants underwent developmental assessments to confirm developmental delays, including evaluations of gross motor, fine motor, speech-language, cognition, social, and emotional domains. The evaluations were performed by members of the early development intervention team at the medical center using the Peabody Developmental Motor Scales, Gross Motor Function Measure, Preschool Language Evaluation Tool, Child Expression Evaluation Tool, Chinese Wechsler Intelligence Scale for Children (3rd edition), and Bayley III Scales of Infant and Toddler Development. A developmental delay was defined as performance two standard deviations or greater below the mean on age-appropriate, standardized, norm-referenced tests. The evaluation team consisted of a physiatrist, a pediatrician, a psychiatrist, an otolaryngologist, an ophthalmologist, physical therapists, occupational therapists, speech therapists, a psychologist, and a social worker. A total of 179 preschool children who were diagnosed with developmental delays were included in the study. In addition, 88 children without developmental delays were recruited from the Department of Pediatrics of Shin Kong Wu Ho-Su Memorial Teaching Hospital to serve as controls. The Research Ethics Committee of the Shin Kong Wu Ho-Su Memorial Teaching Hospital approved the study. Parents or primary caregivers of all the children provided written informed consent before a questionnaire interview and biological specimen collection. This study was performed in accordance with the World Medical Association Declaration of Helsinki.

2.2. Questionnaire interview

Well-trained personnel carried out standardized personal interviews based on a structured questionnaire. The information obtained by the questionnaire included demographics of children and their parents' socioeconomic characteristics.

2.3. Biological specimen collection

Spot urine samples of children and their mothers were collected at the time of recruitment and immediately transferred to a −20 °C freezer and stored until the analysis of arsenic species. We used ethylene-diamine-tetraacetic acid (EDTA) syringes to collect peripheral blood samples from children and their mothers. The samples were centrifuged and the buffy coat from each sample was immediately transferred to a −80°C freezer and stored until DNA extraction for the identification of enzyme gene polymorphisms.

2.4. Urinary arsenic species assessment

Urinary arsenic profiles of As^{III}, DMA^V, MMA^V, and As^V were measured by high-performance liquid chromatography equipped with a hydride generator and atomic absorption spectrometer (HPLC-HG-AAS). The protocol for the determination of the presence of inorganic arsenic and its methylated species was described in a previous study (Hsueh et al., 1998). This method is not influenced by the presence of arsenobetaine and arsenocholine, from seafood, in urine. Our previous study found that frequencies of fish, shellfish, and seaweed dietary intake were not significantly correlated with inorganic arsenic and its methylated species (Hsueh et al., 2002). The recovery rates of As^{III}, DMA^V, MMA^V, and As^V ranged from 93.8 to 102.2%, with detection limits of 0.02, 0.08, 0.05, and 0.07 μg/L, respectively. Freeze-dried SRM 2670