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Global analysis of genetic variation in human arsenic (+3 oxidation state) methyltransferase (AS3MT)

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ABSTRACT

Human arsenic (+3 oxidation state) methyltransferase (AS3MT) is known to catalyze the methylation of arsenite. The objective of this study was to investigate the diversity of the *AS3MT* gene at the global level. The distribution of 18 single nucleotide polymorphisms (SNPs) in *AS3MT* was performed in 827 individuals from 10 populations (Japanese, Korean, Chinese, Mongolian, Tibetans, Sri Lankan Tamils, Sri Lankan Sinhalese, Nepal Tamangs, Ovambo, and Ghanaian). In the African populations, the A allele in A6144T was not observed; the allele frequencies of C35587 were much lower than those in other populations; the allele frequencies of A37616 and C37950 were relatively higher than those in other populations. Among Asian populations, Mongolians showed a different genotype distribution pattern. A lower C3963 and T6144 frequencies were observed, and, in the C37616A and T37950C polymorphism, the Mongolian population showed higher A37616 and C37950 allele frequencies than other Asian populations, similarly to the African populations. A total of 66 haplotypes were observed in the Ovambo, 48, in the Ghanaian, 99, in the Japanese, 103, in the Korean, 103, in the South Chinese, 20, in the Sri Lankan Tamil, 12, in the Sri Lankan Sinhalese, 21, in the Nepal Tamang, 50, in the Tibetan, and 45, in the Mongolian populations. The *D'* values between the SNP pairs were extremely high in the Sri Lankan Sinhalese population. Relatively higher *D'* values were observed in Mongolian and Sri Lankan Tamil populations. Network analysis showed two clusters that may have different origins, African and Asians (Chinese and/or Japanese). The present study is the first to demonstrate the existence of genetic heterogeneity in a world wide distribution of 18 SNPs in *AS3MT*.

Introduction

Arsenic poisoning due to drinking groundwater is a serious problem, particularly in Southeast Asia. Chronic arsenic exposure causes skin, lung, and bladder cancers (IARC, 1980; NRC, 1999) as well as neurotoxicity and hepatic injuries (Simeonova and Luster, 2000). On the other hand, arsenic trioxide is used as an effective medicine for acute promyelocytic leukemia (Douer and Tallman, 2005; Fujihara et al., 2009a).

The toxicity of arsenic greatly differs among their chemical species. Methylated arsenicals had been considered to be less toxic than inorganic arsenic (Yamauchi and Fowler,, 1994). However, it has become obvious that methylation of inorganic arsenic is not necessarily a detoxification process and that methylation is paradoxically both a detoxification and activation process (Thomas et al., 2007). Recent studies have shown that monomethylarsonous acid (MMA^{III}) is more cytotoxic (Petric et al., 2000; Styblo et al., 2000) and genotoxic (Mass et al., 2001) than arsenate and arsenite, suggesting that oxidation state of methylated arsenicals is important to manifest their toxic or genotoxic effects. Methylated and dimethylated arsenic are mainly found as metabolites in human urine (Fujihara et al., 2009a; Crecelius, 1977; Smith et al., 1977). The standard profile is 10–30% inorganic arsenic, 10–20% monomethylated arsenic (MMA: $MMA^{III}+MMA^{V}$), and 60–80% dimethylated arsenic (DMA: $DMA^{III}+DMA^{V}$) (Vahter, 2000; Rossman, 2003). Large inter-individual variability in the arsenic metabolism has been suggested, which may be attributed to the hereditary difference in the enzyme related to the arsenic metabolism (Vahter, 2000).

Arsenic (+3 oxidation state) methyltransferase (AS3MT) catalyzes the methylation of arsenite (Lin et al., 2002). Several single nucleotide polymorphisms (SNPs) in exons and introns in *AS3MT* are reported to be related to inter-individual variation in the arsenic

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metabolism. The M287T polymorphism has been shown to be related to an increased percentage of monomethylated arsenic in urine in central European population (Lindberg et al., 2007) and a Chilean male group (Hernández et al., 2008a, 2008b). Other *in vitro* study has shown that a recombinant 287T variant had increased levels of enzyme activity and immunoreactive protein compared to WT when transfected in COS-1 cells (Wood et al., 2006). Meza et al. (2005) showed that three SNPs in introns (G7395A, G12390C, and T35587C) in the *AS3MT* gene were significantly associated with the urinary dimethylarsenic (V) to monomethylarsenic (V) ratio (D/M ratio). They showed that the T35587C polymorphism is associated with the higher D/M ratio only in Mexican children (Meza et al., 2007). Schläwicke Engström et al. (2007) reported that three intronic SNPs (G12390T, T14215C, and G35991A) were associated with lower percentages of MMA and higher percentages of DMA in urine of Argentines (Schläwicke Engström et al., 2007). As for the Asian population, we have first shown an association of genetic factors with the arsenic metabolism in Vietnamese: the heterozygote for M287T had higher MMA/inorganic arsenic in urine than the homozygote for WT; the homozygote for G 12390 and C 35587 had lower DMA/MMA in urine; the percentage of MMA in urine of the T5913 homozygote was lower than that for the heterozygote (Agusa et al., 2009).

Interestingly, we have previously revealed the Asian-specific low mutation frequencies of the M287T polymorphism in *AS3MT* (Fujihara et al., 2007a, 2007b, 2007c; Fujihara et al., 2008). Moreover, we have reported the ethnic difference in the genotype distribution of five intronic SNPs (G7395A, G12390C, T14215C, T35587C, and G35991A) of the *AS3MT* gene in the Ovambo, Turkish, Mongolian, Korean, and Japanese populations (Fujihara et al., 2009b). Many other SNPs have been reported (Meza et al., 2005). However, the genotype distribution, haplotype analysis, linkage disequilibrium (LD), and other analyses of other SNPs in the *AS3MT* gene in worldwide populations are unknown. Therefore, the purpose of the present study was to document global ethnic variations of allelic frequencies and the patterns that exist in the haplotypes and LD in *AS3MT* polymorphisms. Here, we present data on 18 SNPs, their frequencies, and haplotypes in 827 healthy individuals from 10 Asian and African populations.

Materials and methods

DNA samples. Individuals from ethnically diverse populations were genotyped. Blood or bloodstain samples were randomly collected from unrelated healthy subjects: 102 Ovambos (Bantus in Namibia), 87 Ghanaians (Ghana), 141 Japanese (Shimane and Hokkaido Prefectures in Japan), 230 Koreans (Busan of South Korea), 54 Chinese (Shenyang and Guangzhou of China), 58 Mongolians (Ulaanbaatar in Mongol), 65 Tibetans (Katmandu of Nepal), 31 Tamangs (Kotyang of Nepal), and 29 Sri Lankan Tamils and 30 Sri Lankan Sinhalese (Kandy of SriLanka). Written informed consent was obtained from each participant. The study was approved by the Ethical Committee of Shimane and Kurume University. Genomic DNA was extracted using a QIAamp DNA mini kit (Qiagen, Chatworth, CA). Appropriate institutional review board approval was obtained, and the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations were followed. In addition, for investigations involving human subjects, informed consent was obtained from the participants involved.

Genotyping. The polymorphic site surveyed in this study is shown in Fig. 1. The design of the PCR primers used to detect other SNPs was based upon the nucleotide sequence of human AS3MT (accession number: AY817668). AS3MT T14458C (M287T), G7395A, and G35991A were detected according to our previous methods (Fujihara et al., 2007a, 2007b, 2007c; Fujihara et al., 2009b). A previous detection method of C12390G, T14215C, and T35587C (Fujihara et al., 2009b) was reconstructed in the present study. The mismatched PCR method (Kumar and Dunn, 1989) was employed to create a new restriction enzyme site to detect T4740C, A9749T, and T12590C. The 4740-R, 9749-F, and 12590-R primers were designed with a deliberate mismatch in order to create a new restriction enzyme site (Table 1). Following amplification with PCR, the PCR products were digested with a restriction enzyme as shown in Table 1. Other SNPs were detected using a newly designed PCR-RFLP method. Amplification was performed in a 10-μl reaction mixture containing a GoTaq® Green Master Mix (Promega, Madison, WI, USA). Next, 2 μl of the 10-μl PCR reaction mixture was digested with 1 unit of restriction enzymes (New England Biolabs, MA) at 37 °C for at least 3 h in a final reaction mixture volume of 5 μl. The digested products (5 μl) were then subjected to electrophoresis on an 8% polyacrylamide gel and detected with silver staining according to our previous method (Fujihara et al., 2007a, 2007b, 2007c; Fujihara et al., 2009b). The PCR primers, annealing temperature, and restriction enzymes used in the present study are shown in Table 1. The genotyping was carried out in duplicate. There were no missing data. The nucleotide sequences of the representative subjects of each genotype were confirmed by the dideoxy chain-termination method with the BigDye®Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Urayasu, Japan). The sequence run was performed on a Genetic Analyzer (model 310, Applied Biosystems), and all DNA sequences were confirmed by reading both strands.

Statistical methods. Statistical analysis was carried out using the program STATCEL (OMS Publishing, Inc.). χ^2-analysis was performed to evaluate the Hardy-Weinberg equilibrium. Haplotypes of the SNPs in the *AS3MT* and their frequencies were estimated using PHASE (version 2.1) (Stephens and Donelly, 2003). Linkage disequilibrium (LD) analyses was carried out using the Arlequin (ver 3.11), which is freely available at the following website: http://cmpg.unibe.ch/

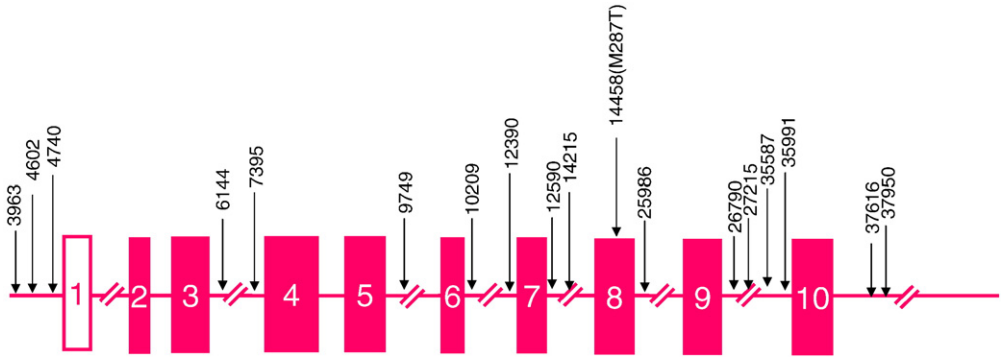

Fig. 1. Human AS3MT polymorphisms studied in this study. Closed rectangles represent coding exons, and white rectangles represent portions of exons that encode the UTR sequence. The number shows the locations of polymorphisms.