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Genetic Polymorphism Influencing Arsenic Metabolism in Human

Haruo TAKESHITA[1], Junko FUJIHARA[1], Tetsuro AGUSA[1,2], Shin TAKAHASHI[2], Hisato IWATA[2] and Shinsuke TANABE[2]

[1]*Department of Legal Medicine, Faculty of Medicine, Shimane University, Enya 89-1, Izumo 693-8501, Japan*
[2]*Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan*

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Abstract—Groundwater pollution by arsenic is a serious worldwide problem, especially in developing Asian and African countries. Large inter-individual variation in the arsenic metabolism has been reported. Human arsenic (+3 oxidation state) methyltransferase (AS3MT) is known to catalyze the methylation of arsenite. The M287T polymorphism in AS3MT is considered to be related to inter-individual variation in the arsenic metabolism. Several genetic polymorphisms within AS3MT genes in the Japanese, Koreans, Chinese, Mongolians, Uygurs, Tibetans, Vietnamese, Tamangs, Tamils, and Sinhalese in Asia and the Ovambos, Ghanaians, and Xhosas in Africa were investigated. Population differences were observed in the only M287T polymorphism in AS3MT. The Asian populations had 287T variant allele frequencies ranging from 0.000 to 0.041, relatively lower than those of the Africans and Caucasians. The other polymorphisms within AS3MT did not show such ethnic differences. Our findings indicate that the genetic susceptibility to arsenic toxicity in Asian populations is different from that in Africans and Caucasians.

Keywords: arsenic metabolism, AS3MT, ethnic differences, genetic polymorphism, PCR-RFLP

INTRODUCTION

Inorganic arsenics are well-known carcinogens of the skin and various internal organs (Fujihara *et al*., 2007a). Groundwater pollution by arsenic is a serious worldwide problem, especially in Asian countries. Higher groundwater concentrations that exceed World Health Organization (WHO) drinking water guidelines (10 ppb) have been reported. Large inter-individual variation has been suggested in the arsenic metabolism (Vahter, 2000), which may be attributed to the genetic polymorphism of the enzyme participating in arsenic methylation. A recent study has shown that monomethylarsonous acid (MMA^{III}) is more cytotoxic and genotoxic than arsenate and arsenite (Petric *et al*., 2000). Arsenic (+3 oxidation state) methyltransferase (AS3MT; previously designated as CYT19) is

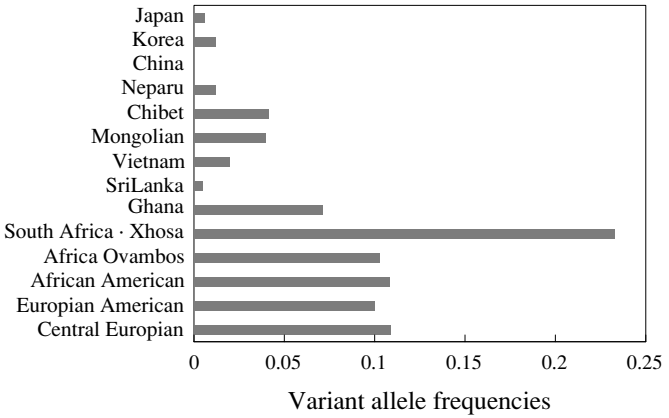

Fig. 1. Comparison of the variant allele frequency of the M287T polymorphism in the AS3MT gene among representative of the examined populations.

an S-adenosyl-L-methionine-dependent enzyme that catalyzes the methylation of arsenite in the rat (Lin *et al.*, 2002). The human AS3MT gene is approximately 32 kb long and is composed of 11 exons, and three exonic single-nucleotide polymorphisms have been shown in African-American and Caucasian-American subjects, i.e., AS3MT: R173W (C517T); M287T (T860C); T306I (C917T) (Wood *et al.*, 2006). Moreover, intronic polymorphisms have also been reported (Wood *et al.*, 2006). In these polymorphisms, a recombinant 287T variant showed increased levels of enzyme activity and immunoreactive protein (Wood *et al.*, 2006). In the central European and Chilean male populations, the 287T variant was shown to increase the percentage of monomethylated arsenic (MMA) in urine (Lindberg *et al.*, 2008; Hernández *et al.*, 2008). These results suggest that the M287T polymorphism in AS3MT may be related to inter-individual variation in the arsenic metabolism. Therefore, genotyping of the M287T and other polymorphisms within the AS3MT gene contributes to understanding genetic susceptibility to arsenic toxicity. Because data for the allele frequencies of the AS3MT polymorphism was limited to few populations (Wood *et al.*, 2006; Lindberg *et al.*, 2008; Hernández *et al.*, 2008), we investigated the allele frequencies for these SNP in 13 populations.

MATERIALS AND METHODS

Samples and DNA isolation

Genomic DNA was extracted from blood or bloodstain samples randomly collected from healthy subjects: 1,074 Japanese, 435 Koreans (Busan of South Korea), 154 Chinese (Shenyang and Guangzhou of China), 246 Mongolians (Ulaanbaatar in Mongol), 56 Uygurs (Urumqi of China), 180 Tibetans (Katmandu of Neparu), 53 Tamangs (Kotyang of Nepal), 190 Vietnamese, 58 Tamils, 54

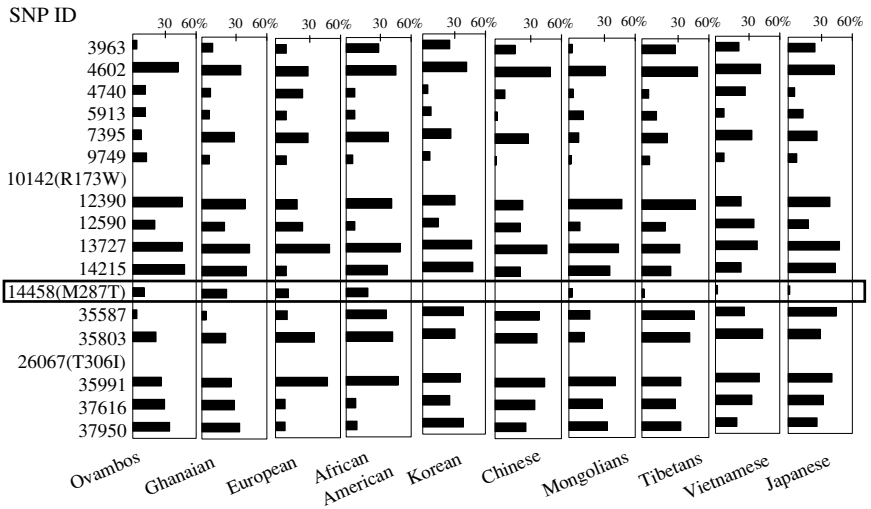

Fig. 2. Variant allele frequencies of the 18 SNPs in the AS3MT gene among representative of the examined populations. SNP ID was taken from Lin *et al*. (2002).

Sinhalese (Kandy of Sri Lanka), 243 Turks (Adana area in Southern Turkey), 120 Ghanaians (Ghana), 185 Ovambos (Bantusin Namibia), and 101 Xhosas (Cape Town of South Africa), which, in part, are the same samples from a previous study (Fujihara *et al*., 2007b, 2008). Informed consent was obtained from each participant. The study was approved by the Ethical Committee of Shimane and Kurume Universities.

Genotyping method of M287T and several polymorphisms in AS3MT

Using the PCR-RFLP method, genotyping of the M287T and 17 other polymorphisms in AS3MT was performed as described previously (Fujihara *et al*., 2007b, 2008, 2009).

RESULTS AND DISCUSSION

In the present study, the allele frequency of the M287T genotypes and other polymorphisms within the AS3MT gene were investigated in 13 populations. The allele frequencies for M287T are shown in Fig. 1. All the genotype distributions were in Hardy-Weinberg equilibrium in all the populations. In the Chinese and Tamil populations, no 287T variant allele was observed. The gene homozygous for the variant 287T was only found in the Xhosa and Ovambo populations. Of 13 populations, Xhosas had the highest variant frequency. Other African and Caucasian populations had similar 287T variant frequencies above 0.100, with the exception of the Ghanaians. On the other hand, the Asian populations had 287T variant allele frequencies ranging from 0.000 to 0.041, relatively lower than

those of the Africans and Caucasians. A significant difference in the variant 287T allele frequencies was observed between Asian (Japanese, Koreans, Chinese, Mongolians, Uygurs, Tibetans, Tamangs, Vietnamese, Tamils, and Sinhalese) and non-Asian populations (Turks, Ovambos, Ghanaians, Xhosas, Caucasian American, Central European, Chilean, and African American) populations (*t*-test, $p < 0.001$). On the other hand, another polymorphism within the AS3MT gene did not show such ethnic differences (Fig. 2). Our findings indicate that genetic susceptibility to arsenic toxicity among Asians differs from that among Africans and Caucasians in only the M287T polymorphism of AS3MT. Further study will be necessary to clarify the role of the M287T polymorphism in the arsenic metabolism, especially among Asian populations. Moreover, further studies (Fujihara *et al*., 2007b; Takeshita *et al*., 2009) are needed to clarify the association between the genetic background and clinical symptoms caused by arsenic exposure and the difference in the arsenic level among ethnic populations.

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H. Takeshita (e-mail: htakeshi@med.shimane-u.ac.jp), J. Fujihara, T. Agusa, S. Takahashi, H. Iwata and S. Tanabe