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SLCO1B1 Variants and Urine Arsenic Metabolites in the Strong Heart Family Study

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Arsenic species patterns in urine are associated with risk for cancer and cardiovascular diseases. The organic anion transporter coded by the gene *SLCO1B1* may transport arsenic species, but its association with arsenic metabolites in human urine has not yet been studied. The objective of this study is to evaluate associations of urine arsenic metabolites with variants in the candidate gene *SLCO1B1* in adults from the Strong Heart Family Study. We estimated associations between % arsenic species biomarker traits and 5 single-nucleotide polymorphisms (SNPs) in the *SLCO1B1* gene in 157 participants, assuming additive genetics. Linear regression models for each SNP accounted for kinships and were adjusted for sex, body mass index, and study center. The minor allele of rs1564370 was associated with lower %MMA (p = .0003) and higher %DMA (p = .0002), accounting for 8% of the variance for %MMA and 9% for %DMA. The rs1564370 minor allele homozygote frequency was 17% and the heterozygote frequency was 43%. The minor allele of rs2291075 was associated with lower %MMA (p = .0006) and higher %DMA (p = .0014), accounting for 7% of the variance for %MMA and 5% for %DMA. The frequency of rs2291075 minor allele homozygotes was 1% and of heterozygotes was 15%. Common variants in *SLCO1B1* were associated with differences in arsenic metabolites in a preliminary candidate gene study. Replication of this finding in other populations and analyses with respect to disease outcomes are needed to determine whether this novel candidate gene is important for arsenic-associated disease risks.

Key Words: **American Indians; arsenic metabolism; arsenic species; SLCO1B1; OATPC; Strong Heart Study.**

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Arsenic exposure is a possible risk factor for diabetes (Maull *et al.*, 2012) and cardiovascular disease (Moon *et al.*, 2012), as well as a known human carcinogen (IARC, 2004). Arsenic metabolism involves a series of methylation and reduction reactions (Thomas, 2009), which may contribute to toxicokinetic variability across individuals (Vahter and Concha, 2001) and varying susceptibility to arsenic health effects (Chen *et al.*, 2003a,b; Huang *et al.*, 2008, 2009). Substantial effort has gone into characterizing the genetic basis of arsenic metabolism processes (Engstrom *et al.*, 2011, 2013; Hernández *et al.*, 2008a,b; Wood *et al.*, 2006) and the diversity of probable metabolic variants across human populations (Fujihara *et al.*, 2007, 2008, 2009, 2010, 2011). More recently, genome-wide screens have been conducted (Pierce *et al.*, 2012; Tellez-Plaza *et al.*, 2013). Less research, however, has focused on the potential role of transporters for arsenic toxicokinetics in humans (Hernández and Marcos, 2008). Transporters play an important role for arsenic kinetics in bacteria (Achour *et al.*, 2007), plants (Briat, 2010; Catarecha *et al.*, 2007; Zhao *et al.*, 2010), fish (Hamdi *et al.*, 2009), amphibians (Villa-Bellosta and Sorribas, 2010), and rodents (Carbrey *et al.*, 2009; Kala *et al.*, 2000; Kojima *et al.*, 2006; Villa-Bellosta and Sorribas, 2008; Wang *et al.*, 2009; Xie *et al.*, 2004). Based on *in vitro* studies in human cells, transporters are also thought to be central to human arsenic toxicokinetics (Calatayud *et al.*, 2010, 2012; Carew and Leslie, 2010; Chavan *et al.*, 2011; Drobná *et al.*, 2010; Lee *et al.*, 2006). Some experiments have also shown the potential of human transporters such as aquaporins to transport arsenic when introduced into other organisms such as yeast (Liu *et al.*, 2002) or frog (McDermott *et al.*, 2010) models. However, given the complexity and context dependence of transporter biology (Kindla *et al.*, 2011; König

et al., 2013; Müller and Fromm, 2011) and the particular challenges for generalizing arsenic findings across toxicological models (States *et al.*, 2011), laboratory results from other models should be regarded as hypothesis-generating for human toxicology. Human data, including genetic epidemiology studies and studies quantifying transporters such as by mass spectrometry (Cutler and Choo, 2011), remain important. Epidemiological studies are needed to clarify the role of transporters for arsenic susceptibility in human populations.

One of the best characterized transporters in pharmacology is the organic anion transporter coded by the Solute Carrier Organic Anion Transporter family member 1B1 (*SLCO1B1*) gene. Perhaps the most clinically important finding is the interaction of rs4149056 with simvastatin that causes myopathy (Wilke *et al.*, 2012), but many other small anionic molecules besides simvastatin are also substrates for this transporter, including arsenicals. The protein coded by *SLCO1B1* has been associated with transport of arsenic in a transformed HEK-293 kidney cell line (Lu *et al.* 2006), but the role of this transporter has not been evaluated in an epidemiological study of arsenic.

The objective of this study was to evaluate the association of variants in *SLCO1B1* with arsenic metabolite patterns in urine of participants from the Strong Heart Study (SHS), a cohort recruited from rural communities of the Southwestern and Midwestern United States exposed to low-to-moderate arsenic levels in drinking water. Epidemiological studies often summarize the pattern of arsenic metabolites in urine as proportions of the 3 main inorganic arsenic metabolites (inorganic arsenic "iAs," monomethylarsonate "MMA," and dimethylarsinate "DMA") contributing to their sum. In the SHS, these % arsenic species appear to be stable within individuals over a 10-year interval (Navas-Acien *et al.*, 2009), highly heritable (Tellez-Plaza *et al.*, 2013), and largely independent of arsenic exposure levels (Tellez-Plaza *et al.*, 2013).

MATERIALS AND METHODS

Study population and measures. The SHS is a prospective cohort study for cardiovascular disease risk factors relevant for American Indian communities from 3 regions: Arizona, Oklahoma, and North and South Dakota (Lee *et al.*, 1990). The study visit included a questionnaire, a physical exam, and biological specimen collection by centrally trained and certified staff (Lee *et al.*, 1990). In the SHS, urine arsenic species were measured in the 1989–1991 baseline visit as a possible cardiovascular disease and diabetes risk factor (Gribble *et al.*, 2012; Scheer *et al.*, 2012). Arsenic species were determined by anion-exchange high-performance liquid chromatography (Agilent 1100, Agilent Technologies, Waldbronn, Germany)-inductively coupled plasma mass spectrometry (Agilent 7700x) and had a detection limit of 0.1 µg/l (Scheer *et al.*, 2012). We excluded 2 participants missing data on total arsenic or arsenic species levels in urine, 222 participants with at least 1 arsenic species below the limit of detection, and 16 participants missing data on body mass index.

The SHS has a genetic component called the Strong Heart Family Study (SHFS), a large pedigree study that includes some of the original SHS participants and their family members (North *et al.*, 2003). Genetic data happened to be available in our candidate gene *SLCO1B1* because a previous study in the SHFS identified a linkage region of interest in Chromosome 12 for left ventricular mass, and subsequent fine mapping of that region genotyped several variants in the *SLCO1B1* gene in SHFS participants. Single-nucleotide polymorphisms (SNPs) were genotyped at the SHFS Genetic Center using the multiplex VeraCode technology from Illumina according to the manufacturer's protocol (Illumina, San Diego, California). Details of the technique are reported elsewhere (Voruganti *et al.*, 2010). Cluster calls were checked for accuracy, and genotypes were exported as text files for further use in association analysis. Replica samples were included as controls for genotyping and allele calling consistency. There was an overlap of 157 SHFS participants with urine arsenic species measures above detection limit, data on correlates of % arsenic species, and *SLCO1B1* genotypes available for this analysis, coming from Arizona, North Dakota, and South Dakota communities. In general, participants in this subset were slightly older, less likely to be current drinkers or smokers, more often female, and had higher blood pressure than in the full SHFS sample recruited from Arizona, North Dakota, and South Dakota communities (see Supplementary data).

Statistical analysis. An allelic association test at each locus, with residual polygenic variance component estimated from family relationships (Boerwinkle *et al.*, 1986), was employed to estimate the associations between the dose of each *SLCO1B1* polymorphism with the mean level and variance of % arsenic species quantitative traits. Genotype frequencies were calculated for each SNP and tested for departures from Hardy-Weinberg equilibrium. Estimates of linkage disequilibrium (LD) between SNPs were determined by calculating pairwise r^2 statistics. We adjusted for the major correlates of arsenic metabolite patterns in this population: Study center, sex, and body mass index. The % arsenic species traits conformed to normal distributions and were not transformed for association analysis. All analyses were performed in the Sequential Oligogenic Linkage Analysis Routines (SOLAR) software (Almasy and Blangero, 1998). We tested for population stratification in each analysis and as a sensitivity analysis estimated a test statistic robust to population stratification, the quantitative transmission disequilibrium test (QTDT) (Abecasis *et al.*, 2000a,b). The QTDT works by partitioning between-family and within-family associations of the genotype with the trait mean; models constraining the within-family association to 0 are compared with models allowing flexible estimation of the within-family association for a test of association that is robust to population stratification because population stratification has minimum influence on within-family comparisons.

RESULTS

Genetic Variants and Phenotypes

Genotype frequencies are shown in Table 1. One participant failed genotyping at reference SNP 2900478 (rs2900478). We calculated the effective number of SNPs accounting for LD to be 4.3 using the method of Moskvina and Schmidt (2008) and therefore set the alpha (adjusting for multiple testing) at .012. The LD patterns for these SNPs are shown in Figure 1; correlations range between .63 and −.24. The % arsenic species biomarker distribution summaries are presented according to genotype in Table 2.

Association Analysis

There were 2 SNPs in *SLCO1B1* that were significantly associated with variability in %MMA and %DMA in additive genetic models after adjusting for study center, sex, and body mass index: rs1564370 and rs2291075 (Table 3). Minor alleles of rs1564370 were associated with lower %MMA and higher %DMA, accounting for 9% of the variance for %DMA

(p = .0002) and 8% for %MMA (p = .0003) (Table 3). The rs1564370 minor allele homozygote frequency was 17% and the heterozygote frequency was 43%. Minor alleles of rs2291075 were associated with lower %MMA and higher %DMA, accounting for 5% of the residual variance for %DMA (p = .0014) and 7% for %MMA (p = .0006) (Table 3). The rs2291075 association with %MMA had evidence for confounding by population stratification. However, the QTDT yielded the same inferences for association as the main analysis (QTDT p value < .001). The nonsignificant association between rs2417955 and %MMA was possibly also confounded by population stratification, but the QTDT again yielded the same inference (p = .017) for an alpha of .012.

TABLE 1
Genotype Frequencies for *SLCO1B1* Polymorphisms

SNP	Genotype	*N*	Frequency	HWE *p* Value
rs1564370	C/C	26	0.17	.90
	C/G	68	0.43	
	G/G	63	0.40	
rs2291075	A/A	2	0.01	.42
	A/G	24	0.15	
	G/G	131	0.83	
rs2417955	A/A	96	0.61	.93
	A/T	56	0.36	
	T/T	5	0.03	
rs2900478	Missing	1	0.01	.71
	A/A	144	0.92	
	A/T	12	0.08	
	T/T	0	< 0.01	
rs4149063	A/A	4	0.03	.90
	A/C	56	0.36	
	C/C	97	0.62	

Note. No markers showed significant departure from HWE. Abbreviation: HWE, Hardy-Weinberg equilibrium.

DISCUSSION

In this preliminary study, common variants in the *SLCO1B1* gene accounted for a substantial amount of phenotypic variance in % arsenic species in urine after controlling for study region, sex, and body mass index. These findings are preliminary evidence in support of the hypothesis that *SLCO1B1* may be an important gene for arsenic toxicokinetics in human populations.

One implication from our study is that the % arsenic species biomarkers commonly used to draw conclusions about arsenic metabolism (Engstrom *et al.*, 2013; Gardner *et al.*, 2011; Li *et al.*, 2011) may reflect genetic influences beyond the assumed metabolic drivers. For example, the most commonly studied arsenic metabolism gene, *AS3MT*, encoding arsenic (III) methyltransferase, is approximately 3018 kb (Smith, 2008; Thorisson *et al.*, 2005; HapMap Data Rel 28 phase II + III, August 10, on NCBI 36 assembly, dbSNP b126) from the *ABCC2* transporter gene whose protein (MRP2) has strong experimental evidence for arsenic transport (Drobná *et al.*, 2010). Also, *AS3MT* is in a conserved haplotype block with a metal transporter gene *CNNM2* (Engstrom *et al.*, 2013; Gomez-Rubio *et al.*, 2010). Recent analyses suggest that haplotypes of this region are associated with DNA methylation in *CNNM2,* which in turn corresponds with expression of *AS3MT* and *CNNM2* (Chen Engstrom *et al.*, 2013), but the relationship of *CNNM2* has not been evaluated yet with urine % arsenic species. Genetic signals ascribed to methylation in some cases might be confounded by genetic

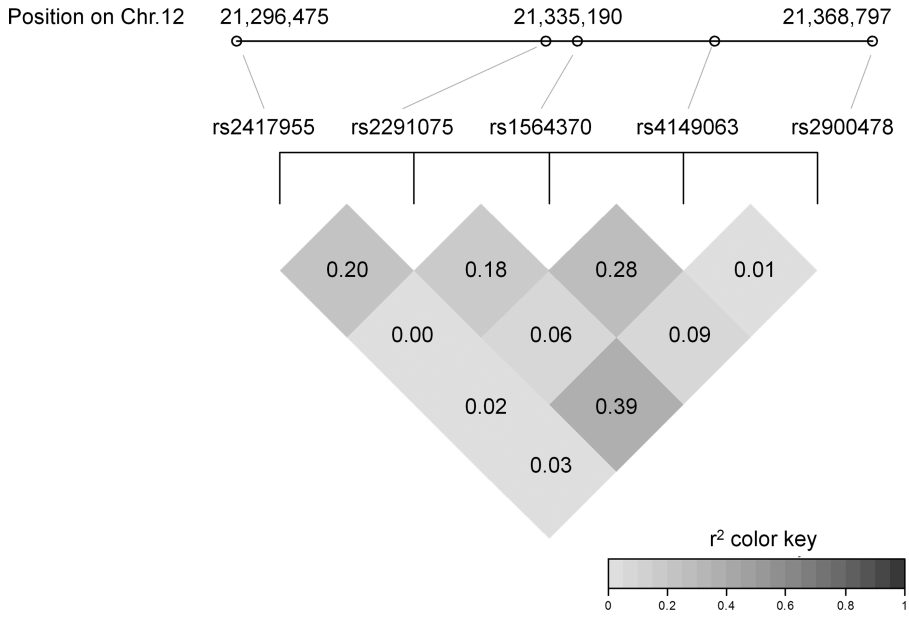

FIG. 1. Linkage disequilibrium (r^2) among SNPs in *SLCO1B1*.

TABLE 2
Urine Arsenic Metabolite Patterns (% Species) by Genotype

SNP	Genotype	Trait	Mean	SD	Minimum	Maximum	*N*
Overall	—	%iAs	8.8	4.9	1.1	31.9	157
	—	%MMA	14.7	5.2	5.1	30.7	157
	—	%DMA	76.4	8.6	31.3	91.5	157
rs1564370	C/C	%iAs	8.2	3.7	3.0	16.9	26
	C/G	%iAs	8.2	4.6	1.1	23.9	68
	G/G	%iAs	9.8	5.6	1.1	38.0	63
	C/C	%MMA	13.9	5.2	7.4	25.4	26
	C/G	%MMA	13.5	4.5	5.1	29.5	68
	G/G	%MMA	16.4	5.4	8.1	30.7	63
	C/C	%DMA	77.9	7.7	61.8	89.5	26
	C/G	%DMA	78.3	7.2	60.7	91.5	68
	G/G	%DMA	73.8	9.7	31.3	87.6	63
rs2291075	A/A	%iAs	6.2	4.4	3.1	9.4	2
	A/G	%iAs	7.4	3.6	2.2	16.9	24
	G/G	%iAs	9.2	5.1	1.1	38.0	131
	A/A	%MMA	8.6	1.6	7.4	9.8	2
	A/G	%MMA	12.3	3.8	5.6	21.2	24
	G/G	%MMA	15.2	5.2	5.1	30.7	131
	A/A	%DMA	85.2	6.1	80.9	89.5	2
	A/G	%DMA	80.3	5.8	66.2	88.6	24
	G/G	%DMA	75.6	8.8	31.3	91.5	131
rs2417955	A/A	%iAs	8.8	4.6	1.1	23.9	96
	A/T	%iAs	9.3	5.6	1.8	38.0	56
	T/T	%iAs	5.9	2.3	3.1	9.4	5
	A/A	%MMA	15.1	4.9	5.8	30.3	96
	A/T	%MMA	14.6	5.5	5.1	30.7	56
	T/T	%MMA	9.1	2.0	7.4	12.2	5
	A/A	%DMA	76.2	7.8	54.8	91.5	96
	A/T	%DMA	76.1	9.8	31.3	88.6	56
	T/T	%DMA	85.1	3.9	80.9	89.5	5
rs2900478	A/A	%iAs	8.9	5.0	1.1	38.0	144
	A/T	%iAs	7.5	4.2	2.2	16.9	12
	A/A	%MMA	15.0	5.2	5.1	30.7	144
	A/T	%MMA	12.2	3.5	7.9	17.2	12
	A/A	%DMA	76.1	8.7	31.3	91.5	144
	A/T	%DMA	80.3	6.8	66.2	87.6	12
rs4149063	A/A	%iAs	11.1	1.7	9.3	12.9	4
	A/C	%iAs	9.7	4.7	1.9	23.9	56
	C/C	%iAs	8.2	5.1	1.1	38.0	97
	A/A	%MMA	17.8	6.3	10.0	25.4	4
	A/C	%MMA	15.0	4.7	6.7	29.5	56
	C/C	%MMA	14.4	5.4	5.1	30.7	97
	A/A	%DMA	71.1	7.8	61.8	80.7	4
	A/C	%DMA	75.3	7.4	60.7	88.3	56
	C/C	%DMA	77.3	9.1	31.3	91.5	97

signals coming from nearby transporters. Underestimating the role of transporters may neglect important exposure-exposure interactions between transporter-affecting chemicals and toxicants (Epel *et al.*, 2008). No previous studies have specifically evaluated the association between *SLCO1B1* variants and arsenic patterns in urine. Understanding the genetic architecture of arsenic susceptibility more comprehensively might provide additional targets for possible interventions to reduce arsenic toxicity. The potential relevance of *SLCO1B1* variation to human population arsenic toxicokinetics does not preclude other transporter genes, perhaps including *CNNM2* (Engstrom *et al.*, 2013) or *AQP3* (Tellez-Plaza *et al.*, 2013), from also being important. The field of arsenic toxicology is ready for further study of transporters (Hernández and Marcos, 2008).

Although the results of this preliminary study are intriguing, our study has several limitations. The sample size was only 157 participants, which was adequate to detect such a major effect with a limited number of hypothesis tests, but may also increase our chance of a false positive. It is important to replicate our finding in other populations, including other participants of the SHFS. Recent research in Northern Mexico has been exploring the genetic epidemiology of arsenic toxicokinetics

TABLE 3
Associations of *SLCO1B1* Genotypes With % Arsenic Species From Measured Genotype Analysis

SNP	Trait	*p* Value	h^2_m	μAA	SE(μAA)	μAB	SE(μAB)	μBB	SE(μBB)	*p* for Population Stratification
rs1564370	%iAs	.010	0.04	12.8	0.9	11.5	0.8	10.2	1.0	1.00
	%MMA	< .001	0.08	17.3	0.9	15.4	0.7	13.5	0.9	.44
	%DMA	< .001	0.09	70.0	1.4	73.3	1.2	76.6	1.6	.89
rs2291075	%iAs	.124	0.01	11.8	0.8	10.3	1.1	8.9	1.9	.61
	%MMA	< .001	0.07	16.0	0.7	12.8	1.1	9.6	1.9	.03
	%DMA	.001	0.05	72.2	1.3	77.3	1.9	82.4	3.2	.24
rs2417955	%iAs	.607	< 0.01	11.8	0.8	11.5	0.9	11.1	1.3	1.00
	%MMA	.185	0.01	16.0	0.8	15.0	0.8	14.1	1.3	.05
	%DMA	.225	< 0.01	72.3	1.4	73.7	1.5	75.1	2.3	.09
rs2900478	%iAs	.092	0.02	11.9	0.8	9.5	1.5	7.2	2.7	.48
	%MMA	.120	0.02	15.8	0.7	13.6	1.5	11.4	2.8	.77
	%DMA	.075	0.02	72.4	1.3	76.7	2.5	81.0	4.7	.84
rs4149063	%iAs	.492	< 0.01	11.4	0.9	11.9	0.8	12.3	1.2	1.00
	%MMA	.317	< 0.01	15.1	0.9	15.9	0.8	16.6	1.3	.76
	%DMA	.372	< 0.01	73.5	1.5	72.4	1.4	71.3	2.1	.58

Note. The h^2_m is the proportion of the residual phenotypic variance explained by the minor allele. Means and standard errors for each % arsenic species biomarker are presented according to genotype, adjusting for study center, sex, and body mass index, along with a *p* value for significance of the association and *p* value for evidence for population stratification. "A" corresponds to the major allele and "B" corresponds to the minor allele for each SNP. Abbreviations: μ, Mean.

(Gomez-Rubio *et al.*, 2010). The comparison of our findings with their study populations, which are more similar in body mass index (Gomez-Rubio *et al.*, 2011) and possibly other attributes relevant for arsenic metabolism than, for instance, populations in Bangladesh (Ahsan *et al.*, 2006; Pierce *et al.*, 2012), would be important. However, we note that the recent genome-wide association scan for urine arsenic species patterns in Bangladesh found an intriguing, but nonsignificant, signal on Chromosome 12 that might reflect the same genetic basis as in our population (Pierce *et al.*, 2012). A second limitation of our study is that it only examined 5 variants in 1 candidate gene. Genotyping additional markers in this region of Chromosome 12 might allow us to better understand the source of the genetic association in future analyses. Neither of the 2 significant SNPs in our study is a known functional mutation. Although we do not know the causal variant, we can speculate about the role *SLCO1B1* might play in the toxicokinetics of arsenic. If this liver uptake transporter affects bilary elimination of arsenic species differentially according to species, then differences in the transporter's function could change the blood distribution of arsenic species, which could affect the arsenic species available to be eliminated at the kidneys into the urine. A third limitation of our study is that we did not measure arsenic species intake or blood arsenic species, so knowledge of the biology of the arsenic transport within the body is limited. However, these limitations do not reduce the potential value of *SLCO1B1* for characterizing susceptibility differences to arsenic across individuals. These are common variants with large estimated effect sizes. It would be useful to examine possible modification of the association of arsenic with clinical outcomes by rs1564370 and rs2291075; even if its biological importance is challenging to clarify, the utility for refining risk assessment for susceptible subpopulations could be major.

In conclusion, this preliminary candidate gene study identified novel, substantial, and significant associations between common *SLCO1B1* variants and the pattern of arsenic metabolites in urine of a subset of participants in the SHFS. Future research is needed to replicate these associations and examine the possible importance for arsenic-associated disease risks.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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