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Arsenic-Gene Interactions and Beta-Cell Function in the Strong Heart Family Study

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Abstract

We explored arsenic-gene interactions influencing pancreatic beta-cell activity in the Strong Heart Family Study (SHFS). We considered 42 variants selected for associations with either beta-cell function (31 variants) or arsenic metabolism (11 variants) in the SHFS. Beta-cell function was calculated as homeostatic model - beta corrected for insulin resistance (cHOMA-B) by regressing homeostatic model - insulin resistance (HOMA-IR) on HOMA-B and adding mean HOMA-B. Arsenic exposure was dichotomized at the median of the sum of creatinine-corrected inorganic and organic arsenic species measured by high performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICPMS). Additive GxE models for cHOMA-B were adjusted for age and ancestry, and accounted for family relationships. Models were stratified by center

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DESCRIPTION OF SUPPLEMENTAL DATA

Supplemental data contain 5 tables.

(Arizona, Oklahoma, North Dakota and South Dakota) and meta-analyzed. The two interactions between higher vs. lower arsenic and SNPs for cHOMA-B that were nominally significant at $P < 0.05$ were with rs10738708 (SNP overall effect -3.91 , $P = 0.56$; interaction effect with arsenic -31.14 , $P = 0.02$) and rs4607517 (SNP overall effect $+16.61$, $P = 0.03$; interaction effect with arsenic $+27.02$, $P = 0.03$). The corresponding genes *GCK* and *TUSC1* suggest oxidative stress and apoptosis as possible mechanisms for arsenic impacts on beta-cell function. No interactions were Bonferroni-significant (1.16×10^{-3}). Our findings are suggestive of oligogenic moderation of arsenic impacts on pancreatic β -cell endocrine function, but were not Bonferroni-significant.

Keywords

genetic epidemiology; environmental epidemiology; susceptibility; arsenicals; diabetes; pancreas

INTRODUCTION

Genetic determinants of pancreatic beta cells' dysfunction and contribution to diabetes pathophysiology are drawing increasing attention (Lawlor *et al.*, 2017). Arsenic is a potential predictor of diabetes (Maull *et al.*, 2012) and related traits including pancreatic beta cell function (Grau-Perez *et al.*, 2017), and has genome-wide epigenetic effects (Bailey and Fry, 2014) which may moderate genetic risk by affecting the regulation of genes relevant to diabetes pathogenesis (Martin *et al.*, 2017). Genetic variation such as in the arsenic (III) methyltransferase gene influences arsenic metabolism (Wood *et al.*, 2006), altering the dose available to exert toxic effects on the pancreas. Limited evidence exists regarding the combined influence of genetic variants and arsenic exposure on pancreatic function. The objective of this study was to explore the joint association of SNPs and arsenic exposure with diabetes-related traits in the Strong Heart Family Study (SHFS).

METHODS

Study Population

The Strong Heart Study [SHS] is a cohort of American Indians from participating tribes and communities in Arizona, Oklahoma, and North Dakota and South Dakota (Lee *et al.*, 1990). Its genetic component, the SHFS, recruited from extended families of SHS participants (North *et al.*, 2003). Details on participant recruitment and information obtained in clinical visits have been published (Lee *et al.*, 1990; North *et al.*, 2003). Baseline recruitment for this study was conducted in two phases [533 participants in 1998–99; 1,941 in 2001–03]. Follow-up visits were conducted in 2001–03, 2005–06 and 2014–15. Inclusion criteria for this analysis were urine arsenic measures, genetic data passing quality control, and absence of diabetes at baseline [$n=1,923$].

Protocols were approved by the Indian Health Service Institutional Review Board, Institutional Review Boards of the participating Institutions, and participating communities. One of the communities [$n=488$] recently opted out of participation in future studies and therefore were excluded from this analysis. All participants provided written informed consent and tribal consent.

Beta-Cell Function, Insulin Resistance, and Incident Diabetes

During follow-up, 256 participants developed incident diabetes defined as fasting blood glucose ≥ 6.99 mmol/L [126 mg/dL], or use of insulin or oral hypoglycemic medications at follow-up visits. Among participants without diabetes, we calculated the homeostatic model - beta corrected for insulin resistance (HOMA-B) using $[20 * \text{fasting insulin in mU/L}] / [\text{fasting glucose in mmol/L} - 3.5]$, and homeostatic model - insulin resistance (HOMA-IR) $[\text{mmol/L}]$ using $[\text{fasting insulin in mU/L} * \text{fasting glucose in mmol/L}] / 22.5$ (Matthews *et al.*, 1985). Since HOMA-B scores are difficult to interpret without taking HOMA-IR into account (Pfaltzner *et al.*, 2010; Yang *et al.*, 2010), we regressed HOMA-B on HOMA-IR and added the mean HOMA-B to the model residual for interpretability based on Willett's nutrient correction method (Willett and Stampfer, 1986). These scores calculated per the method of Willett and Stampfer were called corrected HOMA-B (cHOMA-B) and were used as the primary outcome (S1 Figure).

Measurement of Arsenic Exposure

Spot urine samples were collected in the morning of the baseline visit and frozen and stored at -70°C . Methodology of arsenic measurements have been detailed elsewhere (Scheer *et al.*, 2012). Briefly, concentrations were determined using high performance liquid chromatography-inductively coupled plasma mass spectrometry [HPLC-ICPMS]. Arsenic species levels below limit of quantitation [0.10 $\mu\text{g/L}$] were imputed as the limit of quantitation divided by the square root of 2 [0.07 $\mu\text{g/L}$]. Concentrations of urine inorganic and methylated arsenic species were corrected for creatinine and the sum was taken as a biomarker of inorganic arsenic exposure.

Candidate SNPs

We selected 42 candidate SNPs based on associations with either cHOMA-B [31 SNPs, S1 Table] or arsenic metabolism [11 SNPs, S2 Table] (Balakrishnan *et al.*, 2017) in the SHFS. Genotyping was done with DNA from blood collected at baseline using Illumina Infinium Cardio-Metabo DNA Analysis BeadChip [MetaboChip (Voight *et al.*, 2012) supplemented with a Golden Gate genotyping panel (Illumina) containing 670 candidate SNPs for arsenic metabolism and toxicity. Family-based imputation of genotyped SNPs was done with a PEDSYS-compatible version of Merlin using human genome build 18 [NCBI36/hg18] and 1000 Genomes as the reference panel (Abecasis *et al.*, 2002). Quality control of genetic data excluded participants with call rate $<95\%$, outliers in identity-by-descent [IBD] clustering, or outliers in principal components analysis. We excluded SNPs with minor allele frequency [MAF] $<1\%$, call rate $<98\%$, that were not autosomal, that were not polymorphic, or that violated Hardy-Weinberg equilibrium [HWE] $P < 10^{-5}$.

Statistical Analysis

We tested the hypotheses that there would be heterogeneous pancreatic β -cell function across persons with additive doses of the candidate SNPs and arsenic exposure dichotomized at the median [5.93 $\mu\text{g/L}$] using interaction models. Modeling interactions and describing subgroup effects is appropriate when there are differences in the effects of one variable (e.g., arsenic) according to another variable (e.g., SNPs) (Berrington de Gonzalez and Cox, 2007).

The main outcome of interest was cHOMA-B. Additive genetic models were fit for each candidate SNP, adjusting for age and principal components of genome-wide markers. Models accounted for pedigree structures and stratified by center (Arizona, Oklahoma, and North/South Dakota). Center-specific associations were combined using inverse-variance-weighted meta-analysis. Center-specific analysis was done using SOLAR (Almasy and Blangero, 1998), and meta-analysis using METAL (Willer *et al.*, 2010). We used a nominal P-value threshold of 0.05. The effect Bonferroni-corrected alpha was 1.16×10^{-3} . Among variants with a significant meta-analyzed interaction effect, we also report the main genetic effect from the model without interaction terms. In secondary analyses, we considered log-transformed HOMA-IR scores and incident diabetes as outcomes. For incident diabetes, we performed sensitivity analyses where case definition included glycated hemoglobin thresholds, in addition to analyses excluding controls with impaired fasting glucose.

RESULTS

The median arsenic exposure among participants was 5.93 $\mu\text{g/L}$ (IQR 3.56–9.96); 962 participants were at or below median arsenic exposure and 961 were above median. Median HOMA-B score was 155.2 (IQR 104.9–246) and after correction for HOMA-IR, median cHOMA-B was 186.3 [IQR 153.3–236.2] (Table 1). Among 42 candidate variants from 31 genes, we found suggestive interactions although no associations passed the Bonferroni-corrected significance threshold [S3-S5 Tables]. In particular, two SNPs had nominal interactions with arsenic exposure for c-HOMAB meta-analysis (Table 2): an intergenic SNP rs4607517 [G>A, MAF 0.26], localized to *GCK* and *YKT6* on chromosome 7, and rs10738708 [A>C, MAF 0.43], localized to *TUSC1*.

Among participants with lower than median arsenic exposure, each copy of allele A of rs4607517 was associated with a decrease in cHOMA-B of 35.9 [$P = 2.59 \times 10^{-3}$]. Among participants with higher than median arsenic exposure, the genotype G/G was associated with a decrease in cHOMA-B of 6.1, G/A with a decrease of 14.98, and A/A with a decrease of 23.86. Stratified by center, the expected effect for cHOMA-B scores below median arsenic exposure for allele A was –31.76 for Arizona, –50.59 for Oklahoma, and –32.27 for North/South Dakota (Figure 1, Panel A; S3 Table). The difference in expected cHOMA-B scores comparing above vs. below median arsenic exposure with genotype G/G was +11.94 for Arizona, –22.83 for Oklahoma, and –32.58 for North/South Dakota; with genotype G/A was +29.93, +10.36, and +3.96; and with genotype A/A was +47.92, +43.55, and +40.50 [Figure 1, Panel B; S3 Table]. The overall association of rs4607517 with cHOMA-B, without considering arsenic, was +16.61 per increasing dose of G allele ($P = 0.03$).

For rs10738708, each copy of allele C was associated with an increase in cHOMA-B of 30.47 [$P = 2.74 \times 10^{-3}$] among participants exposed to below median arsenic. Among participants exposed to above median arsenic, genotype AA of rs10738708 was associated with an increase in cHOMA-B of 35.48, AC with an increase of 34.81, and CC with an increase of 34.14. By center, the expected effect for cHOMA-B scores below median arsenic exposure for allele A was –33.20 for Arizona, –60.38 for Oklahoma, and –21.37 for North/South Dakota [Figure 2, Panel A; S3 Table]. The difference in expected cHOMA-B scores comparing above vs. below median arsenic exposure with genotype G/G was –6.49 for

Arizona, -67.22 for Oklahoma, and -39.14 for North/South Dakotas; with genotype G/A was +8.44, -7.66, and -9.86; and with genotype A/A was +23.37, +51.90, and +19.42 (Figure 2, Panel B; S3 Table). The overall association of rs10738708 with cHOMA-B, without considering arsenic, was -3.91 per increasing dose of C allele ($P = 0.56$).

There were two interactions in Arizona and one in the Dakotas that reached nominal significance. In Arizona, rs4139 [A>G, MAF 0.35] localized to *FSTL5* on chromosome 4 and associated with antagonistic interaction effect of +53.44 [$P = 0.03$] [S3 Table]. In addition, rs3740393 [G>C, MAF 0.21], an index SNP for arsenic metabolism localized to *AS3MT* on chromosome 10, had an antagonistic interaction effect of -90.00 [$P = 0.01$] [S3 Table]. In North and South Dakota, there was a nominal antagonistic interaction association for the intergenic SNP rs12642615 [G>A, MAF 0.38] located between *FLJI6686* and *KIAA1239* [32.94, $P = 0.05$].

The interactions between arsenic and SNPs for HOMA-IR scores [S4 Table] and for incident diabetes [S5 Table] were not Bonferroni-significant.

DISCUSSION

We identified several suggestive interactions between arsenic exposure and genetic variants for beta-cell function, although none were significant after accounting for multiple comparisons. We highlight two variants, rs4607517 [*GCK*, *YKT6*] and rs10738708 [*TUSC1*], that had **suggestive interactions** and discuss possible mechanisms below.

The intergenic SNP rs4607517 had an additive allelic effect on cHOMA-B among persons with low arsenic of -35.9 [$P = 2.59 \times 10^{-3}$] and an antagonistic interactive effect (difference in effect for persons with high arsenic) of +27.2 [$P = 0.03$]. Located between *GCK* and *YKT6*, genetic variation in rs4607517 has been associated with BMI and fasting glucose (Dupuis *et al.*, 2010; Manning *et al.*, 2012). Human and animal studies have shown that *GCK* is highly correlated to glucose metabolism, i.e., it is responsible for upregulating glucose-6-phosphatase production via hexokinase in pancreatic beta cells (Osbak *et al.*, 2009). Other variants involved in pancreas development and insulin secretion adversely influence the response to arsenic exposure, with oxidative stress and apoptosis or other cell death mechanisms being top candidate pathways (Hectors *et al.*, 2011).

We found an antagonistic interaction between rs10738708, upstream of *TUSC1*, and arsenic exposure on cHOMA-B although the estimated effects vary by center. The influence of *TUSC1* on pancreatic beta cells or arsenic metabolism is not well established. One possible explanation for this finding **relates** to apoptosis and the rate of cell growth. When *TUSC1* is introduced, tumor cell lines grow slower (Shan *et al.*, 2013); perhaps it also has a role in beta cell development and proliferation. Research in hepatocellular carcinoma cell lines and surgical samples (paired cancer and non-cancer) has suggested that DNA methylation is likely important for regulation of *TUSC1* (Shimizu *et al.*, 2014). The primary rationale for studying arsenic interactions with genes in this study was in part that arsenic is known to affect genome-wide epigenetics (Bailey and Fry, 2014). In this study, among the participants with higher arsenic there were less dramatic differences by *TUSC1* genotype in cHOMA-B,

especially in Oklahoma (Figure 2). However, especially given the limited prior evidence for *TUSC1* in diabetes pathophysiology, and the lack of statistical significance, these findings are tentative.

Our study has several strengths. First, the SHFS has rigorous and consistent data collection among communities affected by both diabetes and arsenic. Second, the complex pedigrees provide increased power to detect genetic effects and consequently interaction effects. Third, correcting HOMA-B scores for insulin resistance allows for a more independent, accurate investigation of beta-cell function compared to raw HOMA-B scores (Pfutzner *et al.*, 2010; Yang *et al.*, 2010). Lastly, although exposure to arsenic is low to moderate, the distribution of arsenic exposure across various centers provides a heterogeneous sample to assess GxE associations.

Our study has limitations. This cross-sectional analysis cannot establish the cause- and-effect relation of the combined influence of genetic variants and arsenic exposure on beta-cell function. Another limitation was the number of participants that developed diabetes over follow-up as well as the number of participants in each genotypic subgroup. To understand the effect of arsenic exposure among different genotypic subgroups, either larger sample sizes or experimental trials reducing exposure to arsenic are required. Finally, we cannot rule out the possibility of unmeasured confounders rather than genotypic subgroups within arsenic exposure categories accounting for observed differences in beta-cell function. For example, if there are other environmental exposures differences that correlate with SNP allele frequencies or with arsenic across these populations, some of the interaction attributed to these SNPs and arsenic could be due to or masked by unmeasured environmental variables. The observed heterogeneity in associations therefore might reflect confounding of the interacting variables. However, it is also possible that unmeasured variables differing between sites, including genetic features, could be higher-order effect modifiers if they impact expression of *TUSC1*, *GCK*, *YKT6* or other biologically important genes, or the biologically effective dose of arsenic.

These exploratory associations may be consistent with oligogenic moderation of arsenic pancreatic endocrine function, but the lack of Bonferroni-corrected statistical significance precludes strong inferences. Since we used a candidate SNP approach focused on common variants, we did not assess the role of rare variation within these pathways or the many other pathways by which arsenic and genes hypothetically could jointly impact pancreatic beta-cell function. Replication in other epidemiological studies and validation through toxicological experiments would strengthen the tentative findings that oxidative stress and apoptosis could be relevant toxicological mechanisms by which arsenic may impact pancreatic function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Genetic variants near tumor suppressor *TUSC1* may alter arsenic pancreatic toxicity.
- Genetic variation near glucokinase *GCK* and secretory protein *YKT6* genes may alter arsenic pancreatic toxicity.
- Additional research on apoptosis and oxidative stress mechanisms is warranted.

Figure 1. Predicted cHOMA-B by rs4607517 Genotype

Expected effect by center for cHOMA-B scores of rs4607517 genotypes (G>A, MAF 0.26) for arsenic exposure below (Panel A) and above (Panel B) median.

Figure 2. Predicted cHOMA-B by rs10738708 Genotype

Expected effect by center for cHOMA-B scores of rs10738708 genotypes (A>C, MAF 0.43) for arsenic exposure below (Panel A) and above (Panel B) median.

Table 1**Baseline Characteristics by Median Arsenic Exposure During Follow-Up**

	At or Below Median (n=962)	Above Median (n=961)	Total (n=1,923)	P-value
Mean age, yrs (SD)	38.2 (16.1)	34.7 (14.8)	36.5 (15.6)	<0.01
No. female (%)	591 (61.4)	566 (58.9)	1,157 (60.2)	0.26
Center (%)				<0.01
Arizona	43 (4.5)	155 (16.1)	198 (10.3)	
Oklahoma	466 (48.4)	353 (36.7)	819 (42.6)	
North/South Dakota	453 (47.1)	453 (47.1)	906 (47.1)	
Median arsenic, µg/L (IQR)	3.6 (2.4–4.7)	10.0 (7.6–15.0)	5.9 (3.6–10.0)	<0.01
Mean BMI, kg/m ² (SD)	29.8 (6.9)	31.0 (7.7)	30.4 (7.3)	<0.01
Mean fasting glucose, mmol/L (SD)	93.2 (9.8)	93.6 (10.7)	93.4 (10.3)	0.39
Mean fasting insulin, pmol/L (SD)	15.6 (15.5)	17.6 (17.2)	16.6 (16.4)	<0.01
Median HOMA-IR (IQR)	2.6 (1.6–4.4)	2.9 (1.8–4.8)	2.7 (1.7–4.6)	<0.01
Median HOMA-B (IQR)	148.4 (100.4–224.2)	165.5 (107.9–264.7)	155.2 (104.9–246)	<0.01
Median cHOMA-B ^a (IQR)	184.7 (153.3–230.1)	187.3 (153.4–240.7)	186.3 (153.3–236.2)	0.35

Abbreviations: BMI, body mass index; HOMA-B, homeostatic model assessment-beta cell function; HOMA-IR, homeostatic model assessment-insulin resistance; yrs, years.

^aCorrected HOMA-B; Sum of residuals adjusted for HOMA-IR and mean HOMA-B.

Table 2

Strongest Interactions of Candidate SNPs with Arsenic for cHOMA-B.

SNP rsID	Chr	Position	Genes	Alleles	MAF	SNP effect among persons with low arsenic		Arsenic effect among major allele homozygote		Interaction	
						Beta	P-value	Beta	P-value	Beta	P-value
rs4607517	7	44202193	<i>GCK, YKT6</i>	G/A	0.26	-35.90	2.59e-3	-6.10	0.50	+27.02	0.03
rs10738708	9	25231316	<i>TUSC1</i>	A/C	0.43	+30.47	2.74e-3	+35.48	0.02	-31.14	0.02

Abbreviations: Chr, chromosome; cHOMA-B, corrected homeostatic model assessment-beta cell function; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

The "Beta" in table refers to estimated difference in means. The notation "e-3" is concise for " $\times 10^{-3}$ ".