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Interaction between arsenic exposure from drinking water and genetic susceptibility in carotid intima-media thickness in Bangladesh

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Abstract

Epidemiologic studies that evaluated genetic susceptibility to the effects of arsenic exposure from drinking water on subclinical atherosclerosis are limited. We conducted a cross-sectional study of 1,078 participants randomly selected from the Health Effects of Arsenic Longitudinal Study in Bangladesh to evaluate whether the association between arsenic exposure and carotid artery intima-medial thickness (cIMT) differs by 207 single-nucleotide polymorphisms (SNPs) in 18 genes related to arsenic metabolism, oxidative stress, inflammation, and endothelial dysfunction. Although not statistically significant after correcting for multiple testing, nine SNPs in *APOE*, *AS3MT*, *PNP*, and *TNF* genes had a nominally statistically significant interaction with well-water arsenic in cIMT. For instance, the joint presence of a higher level of well-water arsenic (> 40.4

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µg/L) and the GG genotype of *AS3MT* rs3740392 was associated with a difference of 40.9 µm (95% CI = 14.4, 67.5) in cIMT, much greater than the difference of cIMT associated with the genotype alone (β = -5.1 µm, 95% CI = -31.6, 21.3) or arsenic exposure alone (β = 7.2 µm, 95% CI = -3.1, 17.5). The pattern and magnitude of the interactions were similar when urinary arsenic was used as the exposure variable. Additionally, the at-risk genotypes of the *AS3MT* SNPs were positively related to proportion of monomethylarsonic acid (MMA) in urine, which is indicative of arsenic methylation capacity. The findings provide novel evidence that genetic variants related to arsenic metabolism may play an important role in arsenic-induced subclinical atherosclerosis. Future replication studies in diverse populations are needed to confirm the findings.

Keywords

arsenic; drinking water; Bangladesh; cardiovascular diseases; carotid artery intima-media thickness; single nucleotide polymorphism

Introduction

Arsenic (As) is a naturally occurring element primarily encountered in drinking water and foods, exposing millions of people in the U.S. and worldwide to this toxic agent. Chronic exposure to As from drinking water has been linked to subclinical and clinical outcomes of cardiovascular disease (CVD) [1]. Carotid artery intima-media thickness (cIMT) is a widely accepted indicator of subclinical atherosclerosis and a valid surrogate marker for clinical endpoints. Epidemiologic evidence suggests a positive association between As exposure and cIMT [2]. In addition, some studies indicate that genetic factors could modify the cardiovascular effects of As exposure [3-7]. However, existing studies have limitations such as a small number of clinical cases of CVD and the inclusion of a limited number of genetic variants. Studies that investigate genetic susceptibility to the cardiovascular effects of As exposure based on subclinical endpoints of CVD, with a more comprehensive genetic approach, are needed. Causal inference between As exposure and CVD can be strengthened if a stronger effect is shown in a genetically susceptible subgroup of the population on subclinical atherosclerosis.

Arsenic in drinking water is present as inorganic As (InAs), either as As^V or As^{III}. As^V is first reduced to As^{III}, followed by methylation to monomethylarsonic acid (MMA^V), which is reduced to MMA^{III}, and at last methylation to dimethylarsinic acid (DMA^V) which can be further reduced to DMA^{III}. MMA is believed to be the more toxic of these metabolites and DMA being more readily excreted in urine and expelled from body [8]. The composition of As metabolites, which often expressed as percentages of all As species in urine (i.e., InAs%, MMA%, DMA%) or as ratios (i.e., MMA/InAs, DMA/MMA), is indicative of methylation efficiency. The enzymatic regulation of As metabolism is partially known. Glutathione-S-transferase omega 1 (GSTO1) catalyzes the reduction of pentavalent As species using glutathione (GSH) as a reducing agent. Other enzymes of GST family, i.e., GST mu 1 (GSTM1), GST pi 1 (GSTP1), and GST theta 1 (GSTT1) play a major role in cellular antioxidant defense mechanisms by catalyzing the reduction of potentially harmful peroxides. Key enzymes involved in the one-carbon methylation of As with S-adenosyl

methionine (SAM) as the methyl donor include arsenic-3-methyltransferase (AS3MT), methylenetetrahydrofolate reductase (MTHFR), cystathionine beta-synthase (CBS), and purine nucleoside phosphorylase (PNP). Genetic polymorphisms in genes encoding above-mentioned enzymes have been related to differences in the distribution of As metabolites in urine [9-18]. Some of these polymorphisms have also been related to CVD risk [16, 19-27]. However, no studies have investigated whether the association between As exposure and subclinical atherosclerosis can be modified by these genetic factors.

Arsenic renders its cardiovascular effects via several potential mechanisms. Experimental studies have suggested that As can induce oxidative stress which may influence gene expression, inflammatory responses, and endothelial nitric oxide homeostasis [28]. These As-induced events may ultimately lead to endothelial dysfunction, which disrupts the balance in vasomotor tone between relaxation and contraction and thus increases CVD risk [29]. Arsenic exposure has also been related to circulating markers of oxidative stress, inflammation, and endothelial dysfunction, such as plasma levels of oxidized low-density lipoprotein, C-reactive protein, soluble intercellular adhesion molecule-1, and soluble vascular adhesion molecule-1 [30-32]. Thus, in addition to genetic variants related to As metabolism, those involved in these mechanisms such as heme oxygenase 1 (*HMOX1*), nitric oxide synthase 3 (*NOS3*), superoxide dismutase 2 (*SOD2*), alpha polypeptide (*CYBA*), apolipoprotein E (*APOE*), tumor necrosis factor (*TNF*), interleukin 6 (*IL6*), intercellular adhesion molecule 1 (*ICAM1*), sphingosine-1-phosphate receptor 1 (*SIP1R1*), and vascular cell adhesion molecule 1 (*VCAM1*) may also modify the cardiovascular effects of As exposure. A few studies in Taiwan have investigated whether As-induced carotid atherosclerosis can be modified by polymorphisms in *HMOX1*, *NOS3*, *SOD2*, *CYBA*, and *APOE* genes [3, 6, 7]. However, larger studies with a comprehensive selection of SNPs are needed to confirm the findings.

We investigated the interaction between As exposure and genetic polymorphisms in 18 genes related to As metabolism [33-37], oxidative stress [38-42], inflammation [41, 43-45], and endothelial dysfunction [30, 31, 46], in cIMT in a cross-sectional study in Bangladesh.

Materials and Methods

Study Population

The parent study, the Health Effects of As Longitudinal Study (HEALS), is an ongoing prospective cohort study in Bangladesh. Details of the study have been presented elsewhere [47]. Briefly, between October 2000 and May 2002, we recruited 11,746 married adults (the original cohort) aged 18 years or more who were residents of the study area for at least 5 years and primarily drinking water from a local tube well. During 2006–2008, the HEALS was expanded to include an additional 8,287 participants (the expansion cohort) following the same methods. The overall participation rate was 97%. At baseline, trained clinicians collected demographic and lifestyle data using a standardized questionnaire, and collected spot urine and blood samples from participants using structured protocols. The cohort is being followed up biennially with similar in-person interviews. Informed consent was obtained from the study participants, and the study procedures were approved by the Ethical

Committee of the Bangladesh Medical Research Council and the institutional review boards of Columbia University and the University of Chicago.

Arsenic exposure measurements

At baseline, water samples from all 10,971 tube wells in the study area were collected. Total As concentration was analyzed by high-resolution inductively coupled plasma mass spectrometry with a detection limit of $< 0.2 \mu\text{g/L}$. Spot urine samples were collected at baseline and at all follow-up visits. Total As concentration was measured by graphite furnace atomic absorption, using a PerkinElmer AAnalyst 600 graphite furnace system (PerkinElmer, Waltham, Massachusetts) with a detection limit of $2 \mu\text{g/L}$ [48]. Urinary creatinine level was analyzed using a method based on the Jaffé reaction [49]. Arsenic exposure concentration might change in some participants since baseline, we thus calculated change in urinary creatinine-adjusted As between visits to tract the change in exposure during follow-up. Urinary As metabolites were measured in baseline urine samples using a method described by Reuter *et al.* [50], as previously described [2, 51].

Measurement of cIMT

A total of 800 participants were randomly selected from the 11,224 original cohort members who provided urine samples at baseline. A total of 700 participants were randomly sampled from the 5,136 participants over 30 years of age in the expansion cohort. In total, cIMT was measured between April 2010 and January 2012 for 1,206 subjects, composed of 600 from the original cohort and 606 from the expansion cohort. The remaining 294 participants did not complete cIMT measurements due to death, move, serious illness, or lack of time. The final study population included 1,078 participants who provided blood samples and thus have genotyping information. The distribution of baseline demographic, lifestyle, and As exposure variables did not differ substantially between the final study population and the overall HEALS (data not shown).

Detailed methods for cIMT measurements have been described previously [2]. Briefly, the measurements were conducted using a SonoSite MicroMaxx ultrasound machine (SonoSite, Inc., Bothell, Washington) equipped with an L38e/10-5-MHz transducer by one designated physician who was blinded to the exposure status of the participants and had extensive training in carotid sonography according to a specific research protocol developed and implemented in the Oral Infections and Vascular Disease Epidemiology Study (INVEST) [52]. cIMT measurements were analyzed offline with MATLAB (MathWorks, Natick, Massachusetts) software, which automatically calculated the distances between the arterial lumen-intima and media-adventitia boundaries and expressed the results as the mean and maximal values. We used the mean of the near and the far walls of the maximum common cIMT from both sides of the neck (mean of 4 sites) as the main outcome variable, similarly to previous studies [53-55].

Selection of genes and single nucleotide polymorphisms (SNPs)

Candidate genes were selected a priori if they 1) are involved in As metabolism, or 2) have been related to As exposure in animal, *in vitro*, or epidemiologic studies and are known to play a key role in CVD risk in epidemiologic studies. We selected 18 candidate genes

related to As metabolism (*GSTM1*, *GSTT1*, *GSTO1*, *GSTP1*, *MTHFR*, *CBS*, *PNP*, and *AS3MT*), oxidative stress (*HMOX1*, *NOS3*, *SOD2*, and *CYBA*), and inflammation and endothelial dysfunction (*APOE*, *TNF*, *IL6*, *ICAM1*, *SIPR1*, and *VCAM1*). We used a comprehensive approach to select SNPs in the candidate genes of interest. We first selected tagSNPs from International Hapmap Project (<http://hapmap.ncbi.nlm.nih.gov/>) and SeattleSNPs (<http://pga.gs.washington.edu/>) using the r^2 -based Tagger program with a pairwise $r^2 \geq 0.80$ and a minor allele frequency (MAF) $\geq 5\%$. The selection was performed for each ethnic group in the Hapmap/SeattleSNPs data separately to compile a list that includes all the tagSNPs. We also selected validated, non-synonymous SNPs with a MAF $\geq 5\%$ from SeattleSNPs and potentially functional SNPs from the F-SNP database (<http://compbio.cs.queensu.ca/F-SNP/>) [56]. To be eligible, selected SNPs were located in exons, exon-intron junctions, promoter regions, or intronic regions that show a $> 80\%$ homology between human and mouse. In addition, we included SNPs that have been related to CVD risk and/or phenotypic markers of interest in the literature.

Genotyping and data cleaning

A total of 384 SNPs with a good illumina score were genotyped using a GoldenGate assay (Illumina, San Diego, CA, USA); 27 SNPs were excluded due to assay failure. A complete list of the 357 SNPs with genotype data is shown in Supplementary Table 1. To ensure quality control, 26 duplicate samples from six subjects were randomly distributed in the genotyping plate. Concordance rates for all assays were $> 99\%$. Of the 357 SNPs, we removed 15 SNPs with call rate $< 95\%$, 79 SNPs with monomorphic genotype data in the overall study population, 10 SNPs with Hardy-Weinberg equilibrium < 0.0001 , and 46 SNPs with an MAF < 0.02 , leaving 207 SNPs in 17 genes for analysis.

Statistical analyses

We first conducted descriptive analyses to assess the associations of demographic, lifestyle, and As exposure variables with cIMT levels defined as quintiles in the overall study population, using Chi-square tests and analysis of variance (ANOVA) for categorical and continuous variables, respectively. We tested interactions between As exposure and each of the SNPs of interest regardless the significance of the main effects of As exposure or SNPs because this approach may capture important interactions limited to a small subset of subjects which result only in weak overall main effect [57, 58]. To test whether the relationship between As exposure and cIMT differed by each SNP, we modeled cIMT as a continuous variable and used linear regression models as following:

$$Y_i = \alpha_0 + \beta_A As_i + \beta_G G_i + \beta_{AG} As_i * G_i$$

where Y_i is cIMT, As_i is As exposure (either well-water As or urinary As) as a continuous variable, G_i is genotypes, corresponding to genotypes under the additive, dominant, or recessive genetic models, and the term $As_i * G_i$ denotes the cross-product of As exposure and a SNP. In the additive genetic model, genotypes of each SNP were coded with values 0, 1, or 2, corresponding to genotypes aa (wild-type homozygote), Aa (heterozygote), and AA (variant homozygote), respectively. In the dominant genetic model, genotypes Aa and AA

were combined and compared to genotype aa, while in the recessive model, genotypes aa and Aa were combined and compared to genotype AA. cIMT was approximately symmetrically distributed in the study, and thus for better interpretation of the results cIMT values were not log-transformed in the analyses. We estimated regression coefficients and their 95% confidence intervals (CIs) for the difference in cIMT in relation to 1) a 1-standard deviation (SD) increase in either baseline well-water As or urinary As (β_A), in the absence of genetic variant, 2) presence of generic variant in the absence of As exposure (β_G), and 3) additional effect of the joint presence of a 1-SD increase in As exposure and generic variant beyond the sum of their individual effects (β_{AG}). The significance of the interaction on the additive level was judged based on the P value for coefficient associated with the cross-product term of each SNP and As exposure (β_{AG}). SNPs that showed nominally statistically significant additive interactions with well-water As in cIMT were further tested for their interactions with urinary creatinine-adjusted As. Potential confounders include sex, age (years), body mass index (kg/m^2), educational attainment (years), smoking status (never, past, and current), systolic blood pressure, and diabetes status (yes/no) at baseline. We additionally adjusted for change in urinary As between visits because it was associated with baseline well-water As status and may be related to health effects [59, 60]. To account for multiple testing, the false-discovery rate (FDR) correction of Benjamini and Hochberg [61] was implemented. However, none of the tested interactions reached the significance threshold (P value < 0.05) after the FDR correction, and therefore the adjusted P values were not shown.

We conducted stratified analyses to further describe the effects of As exposure on cIMT by SNPs that had a nominally statistically significant interaction with both well-water As and urinary As. Well-water As or urinary As was dichotomized based upon the median value in the study population and genotypes were defined in the dominant or recessive genetic model. In addition, in a subset of 829 participants for whom urinary As metabolites had been measured, we computed least squares means of MMA% and DMA% by genotypes of nominally statistically significant SNPs in *AS3MT* defined in the recessive genetic model adjusting for sex, age, body mass index, smoking status (never, past, and current), and educational attainment. FDR correction was conducted using the PROC MULTTEST statement and least squares means were computed using SAS 9.2 (SAS Institute, Cary, NC). All other analyses were performed using R, version 2.13.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Male sex, increasing age, ever smoking among both men and women, and elevated blood pressure were associated with higher levels of cIMT (Table 1). Prevalence of diabetes at baseline was statistically significantly higher in the fifth quintile of cIMT. Average MMA% in urine was higher in participants with higher cIMT. Consistent with our prior data with a smaller sample size [2], there was a positive association between baseline urinary arsenic and cIMT; a 1-SD increase in urinary arsenic ($345.2 \mu\text{g}/\text{g}$ creatinine) was related to a $9.5 \mu\text{m}$ (95% CI: 0.4, 18.6) increase in cIMT after adjustment for potential confounding factors. The positive association between well-water arsenic, albeit not statistically significant in overall (β : $3.0 \mu\text{m}$ for every $96.7 \mu\text{g}/\text{L}$ increase in well-water arsenic; 95% CI: $-2.1, 8.1$), was

statistically significant in those with suboptimal arsenic methylation capacity defined as a higher MMA%, a higher MMA/InAs, or a lower DMA/MMA (data not shown).

Although not statistically significant after correcting for multiple testing, nine SNPs in *APOE*, *AS3MT*, *PNP*, and *TNF* genes had a nominally statistically significant interaction with well-water As in cIMT under at least one genetic model (Table 2). The interactions with *APOE* rs7256173, and *PNP* rs17886095, rs17882804, and rs3790064 were observed under the dominant genetic model whereas the interactions with rs10883790, rs11191442, rs3740392, and rs4919694 in *AS3MT*, as well as *TNF* rs3093661 were present under the recessive genetic model. For instance, cIMT difference was 1.1 μm (95% CI: -4.2, 6.4) for every 96.7 $\mu\text{g/L}$ increase in well-water As in the absence of the AA genotype of rs11191442 in *AS3MT* and -4.8 μm (95% CI: -28.6, 19.0) for the AA genotype in the absence of As exposure. There was an additional increase of 23.8 μm (95% CI: 4.4, 43.2) in cIMT for the joint presence of a higher level of well-water As and the AA genotype beyond the sum of the individual effects. *APOE* rs7256173, and rs10883790, rs11191442, and rs3740392 in *AS3MT* also showed nominally statistically significant interactions with urinary As whereas the SNPs in *PNP* and *TNF* lost nominally statistically significant interactions (Table 3). We therefore further described the joint effects of SNPs in *APOE* and *AS3MT* with As exposure in stratified analyses (Table 4). The linkage disequilibrium (LD) plot for SNPs in *AS3MT* is presented in Supplementary Figure 1. The SNPs of rs10883790, rs11191442, and rs3740392 in *AS3MT* were in high LD, with a pairwise r^2 all > 0.95 for any pair metric.

For SNPs with a nominally statistically significant interaction with As exposure in cIMT, a higher level of well-water As was associated with a greater difference in cIMT among those with the at-risk genotypes, compared to that among those with other genotypes, although the point estimates were not statistically significant (Table 4). For instance, a higher level of well-water As ($> 40.4 \mu\text{g/L}$) was associated with a difference of 32.9 μm (95% CI: -9.8, 75.5) in cIMT among those with the GG genotype of *AS3MT* rs3740392, compared to a difference of 7.6 μm (95% CI: -2.6, 17.8) in cIMT among those with the AA or AG genotype. When single reference group was considered, the joint presence of genetic susceptibility and a higher level of As exposure was associated with a greater difference in cIMT, compared to that for a higher level of As exposure alone or for genetic factor alone. For instance, compared with those with a lower level of well-water As ($< 40.4 \mu\text{g/L}$) and AA or AG genotype, the joint presence of the GG genotype of *AS3MT* rs3740392 and a higher well-water As level was associated with a difference of 40.9 μm (95% CI: 14.4, 67.5) in cIMT. The pattern and magnitude of effect estimates when urinary As was considered in the analyses were consistent. For instance, the joint presence of a higher level of urinary As (183 $\mu\text{g/g}$ creatinine) and the GG genotype of rs3740392 was associated with a difference of 44.1 μm (95% CI = 14.6, 73.6) in cIMT, greater than that associated with the genotype alone ($\beta = 0.4 \mu\text{m}$; 95% CI: -23.3, 24.2) or As exposure alone ($\beta = 5.7 \mu\text{m}$; 95% CI: -4.5, 16.0). However, the effect estimates in relation to a higher level of urinary As and the CT or TT genotype of *APOE* rs7256173 did not reach significance.

Table 5 shows the adjusted means of urinary MMA% and DMA% according to the genotypes of three *AS3MT* SNPs that had nominally statistically significant interactions with both well-water As and urinary As in cIMT. The CC genotype of rs10883790, AA genotype

of rs11191442, and GG genotype of rs3740392 were each statistically significantly associated with elevated MMA% in urine, and marginally statistically significantly related to decreased DMA% in urine, compared with their counterparts without these genotypes, adjusting for sex, age, body mass index, smoking status (never, past, and current), and educational attainment. For instance, participants with the CC genotype of *AS3MT* rs10883790 had an average of 14.3% MMA% and 69.5% DMA% in urine, whereas those with the CA or AA genotype had a statistically significantly lower average of MMA% (12.8%; $P = 0.02$) and higher average of DMA% (71.8%; $P = 0.05$).

Discussion

To the best of our knowledge, the present study is the largest and most comprehensive analysis of interactions between As exposure from drinking water and genetic variants in preclinical measures of CVD. Although none of the interactions displayed statistical significance after adjusting for multiple testing, three SNPs in *AS3MT* showed nominally statistically significant positive interactions with both well-water As and urinary As. In some cases, those with both a higher level of As exposure and the at-risk genotype had a higher cIMT by 40 μm , compared with those without the at-risk genotype and lower level of As exposure. Prior literature has shown that a difference of 100- μm in cIMT being associated with a 50% increased risk of coronary heart disease [62]. Therefore, the present findings indicate that significant gene-As interactions could account for a substantial proportion of CVD risk and be of public health impact. Our findings stress the importance of the removal of arsenic exposure, as it could lead to a more than expected reduction in atherosclerosis due to the existence of gene-arsenic interaction in CVD-related outcomes. Future studies of interaction between arsenic exposure and genetic susceptibility in clinically significant levels of cIMT are needed to confirm the hypothesis.

The effects of *AS3MT* SNPs on As metabolism have been the subject of extensive investigation [63]. Among a number of intronic and exonic variants explored, two SNPs appear consistently associated with altered distribution of urinary As metabolites across populations. The C allele of intronic variant rs3740393 is related to higher urinary DMA/MMA, while the C allele of the other C/T polymorphism (rs11191439), has been related to higher urinary MMA% in Central Europe [14] and Northern Chile [64]. Two studies in Bangladesh including a GWAS conducted in our cohort have identified that eight SNPs including rs1046778, rs11191439, rs3740390, rs9527, rs11191527, rs4919694, rs4290163, and rs11191659 in *AS3MT* are associated with the distribution of urinary As metabolites [9, 10]. A case-control study conducted in Northeastern Taiwan found a synergistic effect between higher As exposure ($> 50\mu\text{g/L}$) and the variant C allele of rs11191439 on carotid atherosclerosis; however, the number of subjects with lower exposure ($< 50\mu\text{g/L}$) and the at-risk genotype was very low ($n = 2$) [4]. In the present study, we included all the 8 SNPs identified by GWAS as well as rs3740393. We found a marginally statistically significant interaction between urinary As and the genotypes of rs11191439 in cIMT in both the additive and dominant genetic models ($\beta = 23.8$, P for interaction = 0.06 and $\beta = 22.9$, P for interaction = 0.08, respectively), as well as an interaction between either well-water As or urinary As and the intronic SNP rs4919694 in cIMT, although the P values were no longer significant after adjusting for multiple comparisons. The low frequency of the variant allele

of these two SNPs (0.056 for rs11191439 and 0.089 for rs4919694) in our study warrants future studies of larger sample size.

We identified three other novel interactions, represented by rs10883790, rs11191442, and rs3740392 in *AS3MT*. These SNPs are in nearly complete linkage with each other, but were not strongly correlated with any of the 8 SNPs identified in our GWAS study as major genetic determinants of As metabolism (Supplementary Figure 1). Importantly, they were positively related to urinary MMA% and negatively related to urinary DMA% (Table 5). It is thus plausible that the effect modification of these SNPs may be through its direct effect on levels of MMA% and DMA% in urine. In this regard, we have previously reported a dose-response relationship between urinary MMA% and cIMT and a more significant association between As exposure and cIMT among subjects with higher urinary MMA% [2]. Collectively, data from the present study and previous studies suggest that individuals genetically predisposed to suboptimal or incomplete As metabolism, as indicated by higher urinary MMA%, are more susceptible to the effect of As exposure on atherosclerosis. However, SNPs that have strongest main effects on As metabolism may be different from the ones that confer significant interactions with environmental factors in atherosclerosis.

Previous studies from Northeastern Taiwan have investigated whether the association between As exposure and carotid atherosclerosis differed by other genes related to As metabolism including *GSTO1*, *GSTP1*, and *PNP* that were also tested in our study. None of these SNPs displayed a statistically significant interaction with As exposure in cIMT in our study. Differences in sample size, arsenic exposure level, selection of SNPs, definition of subclinical atherosclerosis, and genetic background between study populations, as well as false-positive findings due to chance may have contributed to the discrepancy of the data.

Our data also showed some nominally statistically significant interactions between well-water As and SNPs in *APOE* (rs7256173), *PNP* (rs17886095, rs17882804, and rs3790064), and *TNF* (rs3093661). Among these SNPs, only *APOE* rs7256173 also showed a consistent interaction with urinary As. *APOE* plays an important role in removing very low density lipoprotein and chylomicrons as they circulate in the blood after As exposure from drinking water, and it has shown that exposure to a moderate concentration of As increased atheroma formation and vessel wall As accumulation in *APOE*-knockout (*APOE*^{-/-}) mice [45]. The variant identified here is located in the promoter of *APOE* and may have a regulatory function. In contrast, the interactions observed for the SNPs in *PNP* and *TNF* are less likely to represent a biologically functional finding given the lack of a consistent pattern of interactions with urinary As and the lack of data on LD with other SNPs of potentially functional significance in our study population (data not shown).

Strengths of our study include detailed data on As exposure at the individual levels using well water and urine samples with repeated measurements, the use of comprehensive genomic technologies to measure tag SNPs and known/putative functional SNPs, and inclusion of an ethnically homogeneous population which prevents population stratification bias. Our study also has several limitations. Firstly, large-scale interaction studies entail the performance of numerous statistical tests. Evaluating each test at a nominal level of significance would yield a surplus of interactions deemed significant due to chance.

Therefore, we corrected for multiple testing using the FDR approach. However, because of the large number of SNPs analyzed in three genetic models, the FDR-adjusted *P* values for interaction were several orders of magnitude higher than the uncorrected values and none of them reached significance. We thus acknowledge that replication studies in diverse populations are needed to confirm our findings. Secondly, our analyses focused on *a priori* selected SNPs in candidate genes. We therefore cannot exclude possible interactions between As exposure and other SNPs or other genes. The identified SNPs may be markers of the underlying causal variants and their effects could be underestimated if LD is incomplete [65]. Finally, though we did not have data on lipid profiles, we do not believe this would have altered the interpretation of our findings, as available literature does not suggest a positive association between arsenic exposure and lipid profiles, nor was there evidence that the association between arsenic exposure and CVD is modifiable by lipid profiles [66, 67]. cIMT measurements were not conducted for all of the selected participants. However, the distributions of demographic, lifestyle, and arsenic exposure variables in the study population and in the overall cohort were very similar (data not shown). Our study population was exposed to arsenic from drinking water at low-to-moderate levels (mean = 76 µg/L, range 0.1-864 µg/L). We acknowledge that our results might not be generalizable to other populations with low arsenic exposure levels (< 100 or < 50 µg/L) or arsenic exposure from diet such as those encountered in the US. A recent prospective cohort study in the US found a positive association between lower levels of arsenic exposure (< 100 µg/L) and risk of overall and subtypes of CVD [1]. Genetic susceptibility could play a more critical role at low levels of exposure, and whether genetic susceptibility may modify the effect of arsenic at low levels merits future investigation. In addition, it is possible that different genetic susceptibility plays a role in different mechanisms by which arsenic may lead to CVD. Future studies of gene-arsenic interactions are needed for other CVD outcomes.

Conclusions

We found novel evidence of interactions between three genetic variants in As metabolism gene *AS3MT*, including rs10883790, rs11191442, and rs3740392, with both well-water As and urinary As in cIMT. Our findings support the important role of As metabolism in early effects of As exposure on atherosclerosis and also coincide with the notion that genetic alterations by themselves may not substantially impact disease risk, but in concert with environmental exposures may lead to disease. Further mechanistic studies are needed to elucidate the biological basis of the observed interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CI	confidence interval
cIMT	carotid artery intima-media thickness
DMA	dimethylarsinic acid
HEALS	Health Effects of Arsenic Longitudinal Study
InAs	inorganic arsenic
MMA	monomethylarsonic acid
SNP	single nucleotide polymorphism
SD	standard deviation
MAF	minor allele frequency
FDR	false-discovery rate

Highlights

- Nine SNPs had a nominally significant interaction with well-water arsenic in cIMT.
- Three SNPs in *AS3MT* showed nominally significant interactions with urinary arsenic.
- cIMT was much higher among subjects with higher arsenic exposure and *AS3MT* SNPs.
- The at-risk genotypes of *AS3MT* SNPs were positively related to urinary MMA %.

Table 1

Demographic, lifestyle, and arsenic exposure characteristics of participants by quintile of carotid intima-media thickness, Health Effects of Arsenic Longitudinal Study, Bangladesh, 2000–2011^a

	Quintiles of cIMT ^b														P Value ^c
	Total (n = 1078)				1 (n = 192)		2 (n = 192)		3 (n = 192)		4 (n = 192)		5 (n = 192)		
	%	Mean	Median	10th–90th percentile	%	Mean	%	Mean	%	Mean	%	Mean	%	Mean	
cIMT, μm	787.3	768.13	679.8–916.4		672.0		727.0		769.6		822.6		945.3		
Baseline characteristics															
Male sex	39.1				22.9		28.7		39.9		44.7		59.1	<0.001	
Ever smoking															
Men	76.5				61.2		71.0		70.1		82.3		85.0	0.003	
Women	8.7				3.6		7.1		4.6		14.3		19.3	<0.001	
Diabetes	2.1				1.4		0.0		1.4		2.3		5.6	0.001	
Age, years		38.6	38	27–52		31.8		35.4		37.9		42.2		45.8	<0.001
Body mass index ^d		19.9	19.2	16.2–24.4		19.4		19.8		20.2		19.9		20.2	0.068
Education, years		3.0	1	0–9		3.3		2.9		3.1		2.8		3.0	0.608
Systolic blood pressure, mmHg		116.8	115	97–136		111.3		112.9		116.2		118.5		124.9	<0.001
Diastolic blood pressure, mmHg		75.1	74	62–88		73.3		72.9		75.0		76.1		78.4	<0.001
Well water arsenic, $\mu\text{g/L}$		76.4	40.4	1–208		79.0		71.1		78.8		76.1		76.9	0.925
Urinary arsenic, $\mu\text{g/g}$ creatinine		258.7	183	61.0–532.0		245.3		251.1		250.4		241.9		304.8	0.299
MMA%		12.9	12.5	6.9–19.6		11.8		12.6		12.8		13.1		14.4	<0.001
DMA%		71.7	72.5	60.3–81.7		71.5		71.6		72.5		71.5		71.2	0.667
Follow-up characteristics															
Time between baseline and cIMT measurement, years	6.8	8.4		3.6–9.7		7.7		7.0		7.1		6.8		7.3	0.006
Urinary arsenic at first follow-up, $\mu\text{g/g}$ creatinine	212.6	149		54.5–451.4		213.9		212.5		219.2		195.9		221.5	0.711
Urinary arsenic at second follow-up, $\mu\text{g/g}$ creatinine ^e	203.4	149.5		51.0–433.3		202.3		188.0		198.1		205.7		223.7	0.660
Change in urinary arsenic between baseline and the first follow-up	–47.6	–16.7		–225.6–108.5		–31.6		–41.1		–32.7		–47.6		–85.7	0.402

	Quintiles of cIMT ^b												P Value ^c		
	Total (n = 1078)				1 (n = 192)		2 (n = 192)		3 (n = 192)		4 (n = 192)			5 (n = 192)	
	%	Mean	Median	10th—90th percentile	%	Mean	%	Mean	%	Mean	%	Mean		%	Mean
Change in urinary arsenic between the first and second follow-ups ^e	–12.0		–3.5	–143.7–116.0		–16.8		–28.1		–34.4		–2.1		24.4	0.048
Age at cIMT measurement, years	45.5		44.6	33.7–58.0		39.4		42.3		44.6		48.5		52.7	<0.001

Abbreviations: cIMT, carotid intima-media thickness; MMA, monomethylarsonic acid.

^aData were missing on body mass index, systolic blood pressure, diastolic blood pressure, and diabetes status for 2, 3, 4, and 3 subjects, respectively; on baseline well water arsenic for 16 subjects. Data were only available on urinary MMA% for 829 subjects; on urinary arsenic at the first and second follow-ups for only 1064 and 566 subjects, respectively.

^bQuintile 1: 558–704 µm; Quintile 2: 705–748 µm; Quintile 3: 749–793 µm; Quintile 4: 794–860 µm; Quintile 5: 861–1243 µm.

^cP values were computed with the chi-square test or analysis of variance.

^dWeight (kg)/height (m)².

^eIn the original cohort only.

Table 2
Interactions between SNPs and well arsenic in carotid intima-media thickness

Gene	db SNP ID	MAF (%)	Genetic model	β_A^a (95% CI)	β_G^b (95% CI)	β_{AG}^c (95% CI)	<i>p</i> ^d
<i>APOE</i>	rs7256173	T (2.3) ^e	Dominant	1.4 (-3.7, 6.6)	-20.2 (-50.3, 9.9)	49.6 (21.6, 77.6)	0.0005
	rs10883790	C (25.7)	Additive	1.6 (-5.5, 8.7)	1.8 (-8.1, 11.7)	2.0 (-6.1, 10.1)	0.63
			Dominant	3.9 (-3.5, 11.3)	3.7 (-8.8, 16.2)	-2.1 (-11.9, 7.8)	0.68
rs11191442		A (26.0)	Recessive	1.5 (-3.8, 6.8)	-3.6 (-27.5, 20.3)	21.0 (1.0, 41.0)	0.039
			Additive	0.9 (-6.2, 8.0)	0.9 (-8.9, 10.8)	2.9 (-5.1, 10.9)	0.47
			Dominant	3.6 (-3.8, 11.0)	2.7 (-9.7, 15.2)	-1.6 (-11.4, 8.2)	0.75
			Recessive	1.1 (-4.2, 6.4)	-4.8 (-28.6, 19.0)	23.8 (4.4, 43.2)	0.016
			Additive	0.8 (-6.5, 8.1)	1.4 (-8.5, 11.3)	2.8 (-5.2, 10.9)	0.49
rs3740392		G (26.2)	Dominant	3.7 (-3.9, 11.3)	3.1 (-9.3, 15.6)	-1.8 (-11.7, 8.1)	0.73
			Recessive	1.1 (-4.2, 6.4)	-3.0 (-26.6, 20.6)	23.6 (4.2, 43.1)	0.017
			Additive	1.2 (-4.3, 6.8)	1.2 (-13.4, 15.8)	8.5 (-2.8, 19.8)	0.14
rs4919694		C (8.9)	Dominant	1.8 (-3.8, 7.4)	2.5 (-14.0, 19.0)	6.3 (-6.8, 19.4)	0.34
			Recessive	2.2 (-3.0, 7.4)	-6.3 (-58.3, 45.7)	40.4 (3.4, 77.4)	0.032
			Additive	2.1 (-3.1, 7.2)	-8.8 (-41.8, 24.1)	35.0 (4.1, 65.9)	0.027
			Dominant	1.4 (-4.2, 7.0)	1.5 (-10.0, 13.0)	8.9 (-0.8, 18.6)	0.071
			Additive	0.7 (-4.9, 6.4)	-2.6 (-15.9, 10.8)	13.5 (1.6, 25.4)	0.026
rs3790064		G (4.1) ^e	Recessive	3.2 (-2.0, 8.4)	26.6 (-9.2, 62.5)	-0.4 (-27.6, 26.9)	0.98
			Dominant	2.0 (-3.2, 7.3)	-6.4 (-30.0, 17.3)	24.7 (0.6, 48.7)	0.044
			Additive	3.7 (-2.0, 9.3)	3.3 (-11.8, 18.3)	-4.0 (-15.2, 7.2)	0.48
rs3093661		A (10.0)	Dominant	3.0 (-2.7, 8.7)	0.8 (-15.3, 16.9)	-0.9 (-13.4, 11.7)	0.89
			Recessive	3.6 (-1.6, 8.8)	51.5 (-18.1, 121.1)	-47.3 (-90.4, -4.2)	0.032

Abbreviations: SNPs, single nucleotide polymorphisms; CI, confidence interval; cIMT, carotid intima-media thickness.

^a Coefficient in relation to a 1-standard-deviation increase in well arsenic (96.7 µg/L).

^b Coefficient in relation to each SNP in the additive, dominant, or recessive genetic model.

^c Coefficient in relation to multiplicative interaction between a 1-standard-deviation increase in well arsenic and each SNP.

^d Raw *P* values for multiplicative interaction adjusted for sex, age at cIMT measurement, body mass index, smoking status (never, past, and current), educational attainment, systolic blood pressure, diabetes status at baseline, and change in urinary arsenic level between visits.

^e Analyses were not performed on the additive or recessive scale due to very few number of variant genotype.

^f Deletion/insertion variation.

Table 3
Interactions between SNPs and urinary arsenic in carotid intima-media thickness

Gene	db SNP ID	MAF (%)	Genetic model	β_A^a (95% CI)	β_C^b (95% CI)	β_{AC}^c (95% CI)	<i>p</i> ^d
<i>APOE</i>	rs7256173	T (2.3) ^e	Dominant	7.3 (-1.9, 16.6)	-19.0 (-54.7, 93.2)	50.3 (7.5, 93.2)	0.021
	rs10883790	C (25.7)	Additive	6.2 (-4.0, 16.5)	-0.9 (-11.5, 9.6)	6.3 (-3.8, 16.5)	0.22
	rs111191442	A (26.0)	Dominant	8.1 (-2.2, 18.4)	0.5 (-12.7, 13.7)	2.6 (-9.3, 14.5)	0.67
			Recessive	7.0 (-2.3, 16.2)	-15.0 (-42.3, 12.4)	40.9 (10.6, 71.3)	0.008
			Additive	5.0 (-5.3, 15.2)	-1.4 (-12.0, 9.1)	6.9 (-3.3, 17.0)	0.18
			Dominant	6.7 (-3.7, 17.1)	-0.7 (-13.9, 12.4)	3.5 (-8.5, 15.4)	0.57
	rs3740392	G (26.2)	Recessive	6.0 (-3.3, 15.2)	-13.1 (-40.2, 14.0)	40.4 (10.2, 70.6)	0.009
			Additive	6.4 (-3.8, 16.6)	-0.4 (-11.0, 10.1)	6.3 (-4.0, 16.7)	0.23
			Dominant	8.2 (-2.1, 18.5)	0.5 (-12.6, 13.7)	2.4 (-9.6, 14.3)	0.69
			Recessive	6.9 (-2.2, 16.1)	-15.2 (-42.9, 12.5)	47.6 (14.9, 80.2)	0.004
	rs4919694	C (8.9)	Additive	7.8 (-1.6, 17.2)	-0.4 (-17.1, 16.2)	12.2 (-4.7, 29.0)	0.16
			Dominant	8.2 (-1.2, 17.7)	0.9 (-17.6, 19.4)	10.5 (-9.0, 29.9)	0.29
			Recessive	8.4 (-0.7, 17.5)	-18.0 (-82.3, 46.2)	50.4 (-7.2, 108.0)	0.086
			Dominant	9.3 (0.1, 18.4)	21.2 (-12.1, 54.6)	-6.7 (-34.6, 21.3)	0.64
<i>PNP</i>	rs17886095	A (2.1) ^e	Additive	5.8 (-4.3, 15.9)	-0.2 (-12.0, 11.7)	9.6 (-0.9, 20.1)	0.072
	rs17882804 ^f	I (16.9)	Dominant	6.5 (-3.6, 16.6)	-0.5 (-13.6, 12.7)	8.3 (-2.6, 19.1)	0.14
			Recessive	8.5 (-0.6, 17.7)	-16.9 (-66.1, 32.4)	65.4 (6.2, 124.6)	0.030
			Dominant	9.2 (-0.03, 18.4)	8.3 (-16.7, 33.4)	0.6 (-22.2, 23.4)	0.96
<i>TNF</i>	rs3790064	G (4.1) ^e	Additive	9.3 (-0.1, 18.7)	-0.2 (-17.2, 18.4)	0.6 (-17.2, 18.4)	0.95
	rs3093661	A (10.0)	Dominant	9.3 (-0.1, 18.7)	0.2 (-17.9, 18.3)	0.3 (-17.9, 18.6)	0.97
			Recessive	9.3 (0.2, 18.5)	-11.4 (-10.5, 82.3)	14.8 (-126.4, 156.0)	0.84

Abbreviations: SNPs, single nucleotide polymorphisms; CI, confidence interval; cIMT, carotid intima-media thickness.

^a Coefficient in relation to a 1-standard-deviation increase in urinary arsenic (345.2 µg/g creatinine).

^b Coefficient in relation to each SNP in the additive, dominant, or recessive genetic model.

^c Coefficient in relation to multiplicative interaction between a 1-standard-deviation increase in urinary arsenic and each SNP.

^d Raw *P* values for multiplicative interaction adjusted for sex, age at cIMT measurement, body mass index, smoking status (never, past, and current), educational attainment, systolic blood pressure, diabetes status at baseline, and change in urinary arsenic level between visits.

^e Analyses were not performed on the additive or recessive scale due to very few number of variant genotype.

^f Deletion/insertion variation.

Table 4
Carotid intima-media thickness in relation to joint selected SNPs and arsenic exposure

Arsenic exposure ^a	Genotypes	<i>n</i>	β (95% CI) ^{b, c}	β (95% CI) ^{b, d}
Well arsenic				
<i>APOE</i> (rs7256173)				
< 40.4	CC	504	Reference	Reference
40.4	CC	504	8.8 (-1.2, 18.8)	8.7 (-1.4, 18.8)
< 40.4	CT+TT	25	Reference	-8.1 (-40.4, 24.1)
40.4	CT+TT	21	53.8 (-12.0, 119.6)	46.0 (10.7, 81.3)
<i>AS3MT</i> (rs10883790)				
< 40.4	CA+AA	490	Reference	Reference
40.4	CA+AA	483	8.3 (-1.9, 18.6)	8.0 (-2.3, 18.3)
< 40.4	CC	37	Reference	-5.4 (-32.2, 21.4)
40.4	CC	38	25.2 (-17.8, 68.2)	35.8 (9.2, 62.4)
<i>AS3MT</i> (rs11191442)				
< 40.4	TT+TA	488	Reference	Reference
40.4	TT+TA	479	8.1 (-2.1, 18.4)	7.8 (-2.5, 18.1)
< 40.4	AA	37	Reference	-5.8 (-32.5, 21.0)
40.4	AA	39	29.2 (-13.9, 72.4)	38.3 (12.1, 64.5)
<i>AS3MT</i> (rs3740392)				
< 40.4	AA+AG	489	Reference	Reference
40.4	AA+AG	487	7.6 (-2.6, 17.8)	7.2 (-3.1, 17.5)
< 40.4	GG	38	Reference	-5.1 (-31.6, 21.3)
40.4	GG	38	32.9 (-9.8, 75.5)	40.9 (14.4, 67.5)
Urinary arsenic ^a				
<i>APOE</i> (rs7256173)				
< 183	CC	508	Reference	Reference
183	CC	514	8.1 (-1.9, 18.1)	7.9 (-2.2, 18.1)
< 183	CT+TT	26	Reference	7.5 (-24.2, 39.2)
183	CT+TT	22	15.6 (-53.8, 84.9)	27.9 (-6.6, 62.4)
<i>AS3MT</i> (rs10883790)				
< 183	CA+AA	486	Reference	Reference
183	CA+AA	501	6.2 (-5.1, 16.4)	5.9 (-4.4, 16.2)
< 183	CC	46	Reference	-2.9 (-27.2, 21.3)
183	CC	31	34.0 (-10.9, 78.8)	40.7 (11.6, 69.9)
<i>AS3MT</i> (rs11191442)				
< 183	TT+TA	483	Reference	Reference
183	TT+TA	498	5.9 (-4.3, 16.1)	5.7 (-4.6, 16.0)
< 183	AA	47	Reference	-0.2 (-24.1, 23.8)
183	AA	31	31.8 (-13.7, 77.4)	40.4 (11.4, 69.5)
<i>AS3MT</i> (rs3740392)				
< 183	AA+AG	484	Reference	Reference
183	AA+AG	506	6.0 (-4.2, 16.2)	5.7 (-4.5, 16.0)

Arsenic exposure ^a	Genotypes	<i>n</i>	β (95% CI) ^{b, c}	β (95% CI) ^{b, d}
< 183	GG	48	Reference	0.4 (-23.3, 24.2)
183	GG	30	33.7 (-11.4, 78.9)	44.1 (14.6, 73.6)

^a Cutoff points were determined by median values.

^b Results were adjusted for sex, age at cIMT measurement, body mass index, smoking status (never, past, and current), educational attainment, systolic blood pressure, diabetes status at baseline, and change in urinary arsenic level between visits.

^c Stratified analyses.

^d Joint effects.

^e Deletion/insertion variation.

Table 5
Relationship between selected *AS3MT* SNPs and urinary arsenic metabolites

	MMA %			DMA %		
	<i>n</i>	Adjusted mean (SD) ^a	<i>P</i>	Adjusted mean (SD) ^a	<i>P</i>	<i>P</i>
rs10883790						
CA+AA	766	12.8 (0.17)	0.02	71.8 (0.30)		0.05
CC	55	14.3 (0.62)		69.5 (1.13)		
rs11191442						
TT+AT	761	12.8 (0.17)	0.03	71.9 (0.30)		0.07
AA	56	14.2 (0.62)		69.8 (1.11)		
rs3740392						
AA+AG	769	12.8 (0.17)	0.04	71.8 (0.30)		0.10
GG	56	14.1 (0.62)		69.9 (1.12)		

^aMeans were adjusted for sex, age at cIMT measurement, body mass index, smoking status (never, past, and current), and educational attainment.