Mendelian randomization of inorganic arsenic metabolism as a risk factor for hypertension- and diabetes-related traits among adults in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) cohort

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Abstract

Background: Hypertension and diabetes have been associated with inefficient arsenic metabolism, primarily through studies undertaken in populations exposed through drinking water. Recently, rice has been recognized as a source of arsenic exposure, but it remains unclear whether populations with high rice consumption but no known water exposure are at risk for the health problems associated with inefficient arsenic metabolism.

Methods: The relationships between arsenic metabolism efficiency (% inorganic arsenic, % monomethylarsenate and % dimethylarsinate in urine) and three hypertension- and seven diabetes-related traits were estimated among 12 609 participants of the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). A two-sample Mendelian randomization approach incorporated genotype-arsenic metabolism relationships from literature, and genotype-trait relationships from HCHS/SOL, with a mixed-effect linear model. Analyses were stratified by rice consumption and smoking.

Results: Among never smokers with high rice consumption, each percentage point increase in was associated with increases of 1.96 mmHg systolic blood pressure ($P = 0.034$) and 1.85 mmHg inorganic arsenic diastolic blood pressure ($P = 0.003$). Monomethylarsenate was associated with increased systolic (1.64 mmHg/percentage

point increase; $P = 0.021$) and diastolic (1.33 mmHg/percentage point increase; $P = 0.005$) blood pressure. Dimethylarsinate, a marker of efficient metabolism, was associated with lower systolic (−0.92 mmHg/percentage point increase; $P = 0.025$) and diastolic (-0.79 mmHg/percentage point increase; $P = 0.004$) blood pressure. Among low rice consumers and ever smokers, the results were consistent with no association. Evidence for a relationship with diabetes was equivocal.

Conclusions: Less efficient arsenic metabolism was associated with increased blood pressure among never smokers with high rice consumption, suggesting that arsenic exposure through rice may contribute to high blood pressure in the Hispanic/Latino community.

Key words: Arsenic, arsenic methylation, arsenic metabolism, cardiovascular disease, hypertension, blood pressure, diabetes, Mendelian randomization, Hispanic/Latino health

Key Messages

- Never smokers who are poor metabolizers of arsenic face an increased risk of hypertension.
- The association was observed for those with levels of arsenic exposure that can be acquired through diets high in rice.
- Future research should examine other populations with high levels of rice consumption, who may also be at increased risk.
- Evidence for an association between arsenic metabolism and diabetes-related traits was equivocal.

Introduction

The health consequences of inorganic arsenic (iAs) have largely been identified from populations who were exposed to arsenic through drinking water. In these studies, water arsenic concentrations typically exceed the WHO recommended level of 10 μg/L.[1–5] Chronic exposure to elevated levels of iAs is associated with increased risk of hypertension and diabetes.[6–8] Furthermore, risk is further related to differences in the ability to metabolize iAs.[1,2] Metabolism of iAs is accomplished through a series of reduction, oxidation and methylation reactions.[9] During this process, the most abundant intermediate metabolite is monomethylarsenate (MMA), which can then be further methylated to produce dimethylarsinate (DMA).[9] Among individuals with the same level of iAs exposure, inefficient metabolizers (higher % iAs and % MMA in their urine) have been found to have higher risk of arsenic-associated disease, and efficient metabolizers (higher % DMA) typically have lower risk.[10,11]

Rice, a staple of many Hispanic/Latino diets,[12] has recently been recognized as a significant contributor of dietary iAs. Previous research has found that in populations with no known water exposure but high rice consumption, biomarkers of internal arsenic exposure approach those of populations with water exposure above 10 μg/L.[13–16] This suggests that within communities with rice-centred diets, poor arsenic metabolizers may be at increased risk of arsenic-associated diseases. However, few studies have measured iAs metabolism in populations without water exposure, making direct assessment of this hypothesis challenging.

In populations in which urinary arsenic metabolites have not been directly measured but genotypes have, it is possible to estimate the influence of iAs metabolism using a two-sample Mendelian randomization.[17,18] This approach incorporates previous research that has identified multiple single nucleotide variants (SNVs) that affect iAs metabolism.[19–22] These are combined with study-specific SNV-outcome associations to estimate iAs metabolism-outcome relationships.

This study estimates the relationship between iAs metabolism and hypertension- and diabetes-related traits in participants of the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) by implementing a two-sample Mendelian randomization approach, stratifying by rice consumption and smoking status.

Methods

Study population

The Hispanic Community Health Study/Study of Latinos (HCHS/SOL) is a community-based cohort study of Hispanic and Latino adults in four cities in the USA:

Chicago, Miami, the Bronx and San Diego. The study design has been described previously.[23] This analysis included the 12 609 HCHS/SOL participants with complete genotype data and active consent.

Assessment of hypertension- and diabetes-related traits

Hypertension- and diabetes-related traits were assessed at baseline, as described elsewhere.[24] Systolic and diastolic blood pressures were measured three times with a tested automated sphygmomanometer (Omron model HEM-907 XL; Omron Healthcare Inc., Bannockburn, IL) with seated participants. The average was recorded. Pulse pressure was calculated as the difference between systolic and diastolic blood pressures. For individuals treated with blood pressure-lowering medication, the following adjustments to the observed values were made: systolic + 10 mmHg and diastolic + 5 mmHg.[25]

Fasting glucose, insulin, triglycerides and HbA1c were measured at the baseline interview from blood collected after an 8-h fast. For individuals treated with diabetic medication, the following adjustments were made: fasting glucose + 30 mg/dl, fasting triglycerides + 30 mg/dl and HbA1c + 1.5%.[26] Three diabetes-related indices were calculated: homeostatic model assessment of beta cell function (HOMA-B),[27] homeostatic model for insulin resistance (HOMA-IR)[27] and McAuley insulin sensitivity index.[28] Thirty participants did not fast; their glucose, insulin and triglycerides were set to missing. A small number of participants were outliers; these participants were top-coded at the following levels: fasting glucose (400 mg/dl, 10 participants), HbA1c (15%, eight participants), HOMA-IR (100 mg/dl·mU/L, 17 participants), HOMA-B [2500 (mU/L)/(mg/dL), six participants], fasting insulin (200 mU/L, six participants) and fasting triglycerides (2000 mg/dl, one participant).

Classifying rice consumption

The HCHS/SOL participants completed two 24-h recalls of their food intake; the first at enrolment, and the second within 1 month of the baseline interview.[29] Three questions queried the number of servings of grains, flour and dry mixes consumed (pasta and corn tortillas were assessed separately). These questions could have referred to consumption of amaranth, barley, buckwheat, corn flour, millet, oats, rye, sorghum, spelt, teff, triticale, quinoa and wheat flour as well as rice. Comparisons with the food propensity questionnaire (Supplementary Figure 1, available as Supplementary data at *IJE* online) confirm that rice is the primary grain. The total number of servings of grain from the two 24-h recalls were averaged. The top decile of grain consumers averaged more than 4.7 servings of grains ($n = 1261$ high consumers).

Identifying variants associated with arsenic metabolism efficiency

PubMed was searched to identify studies that investigated genetic associations with arsenic metabolism efficiency. The study was considered if arsenic metabolism efficiency was measured linearly as % iAs, % DMA, and % MMA, and a variant met the customary threshold for genome-wide significance ($P < 5{\cdot}10^{-8}$). Three SNVs in two studies met these criteria (Table 1), with all of the studies undertaken in populations of South Asian ancestry.[20,21] Two of the SNVs are located in chromosome 10 near the region of *AS3MT*: rs9527 and rs11191527.[20,30] The third, rs61735836,[21] is located within a coding region of *FTCD* on chromosome 21.

Smoking status

Smoking has a complex and inconsistent relationship with blood pressure in population-level studies.[31–34] In order to avoid this complicating the interpretation, the analysis was stratified by ever- and never-smoking status ($n = 5074$ and 7535, respectively), as assessed during the baseline interview.

Genotyping in HCHS/SOL

The genotyping and quality control for the HCHS/SOL participants are described elsewhere.[35] Briefly, DNA was extracted from blood and genotyped on an Illumina custom array expanded from the Illumina Omni 2.5 M array (HumanOmni2.5-8v1-1). Samples were excluded for sex mismatch, gross chromosomal anomalies, duplicates, high missing call rates, and evidence of contamination or batch effects. A total of 12 689 samples passed quality control and had active consent at the time of analysis. SNVs were excluded for high missing call rates, Mendelian errors, duplicate-sample discordance and deviation from ancestry-specific Hardy-Weinberg equilibrium ($P < 10^{-5}$).

One of the three variants identified above was directly measured by the array (rs11191527). The other two were imputed to the 1000 Genomes Project phase 1 reference panel (OEvar/r^2 = 1 for both).[36] SHAPEIT2 (v.2.r644)[37] pre-phased and IMPUTE2 (v.2.3.0)[38] implemented the imputation. The SNVs were converted to forward strand and the effect alleles were harmonized with published literature as recommended in Hartwig *et al.*[39]

Statistical methods for SNV-trait associations within HCHS/SOL

To improve normality, HOMA-IR, HOMA-B, McAuley index, fasting insulin and fasting triglycerides were log-

Table 1. Effect sizes and standard errors for SNV-arsenic metabolism efficiency relationships found in published literature

			% iAs		% MMA		% DMA	
rs number	Chr: pos	Eff/ref	Beta	SE	Beta	SE	Beta	SE
rs9527[9,22]	10: 104623578	T/C	1.81	0.37	2.01	0.28	−3.82	0.48
rs11191527[9,22]	10: 104795134	C/T	1.32	0.27	0.98	0.20	−2.30	0.35
rs61735836[23]	21: 47572887	A/G	2.71	0.37	2.42	0.29	−5.09	0.51

Chr: pos: chromosome and position. Position locations refer to the hg19 assembly.

% iAs, percentage of inorganic arsenic; % MMA, percentage of monomethylarsenate; % DMA, percentage of dimethylarsinate; eff/ref, the effect and reference allele from the literature; SE, standard error.

transformed before analysis. The relationships between the three Mendelian randomization SNVs and the hypertension- and diabetes-related traits were calculated using a mixed-effect linear model[35] using the GENESIS R package.[40,41] Mixed effects were used to control for kinship, household and block group; fixed effects were used to control for the top five principal components, genetically-ascertained ancestry group[42] and the log of the sampling weights. The analyses were stratified by rice consumption and smoking status.

Statistical methods for Mendelian randomization

In the HCHS/SOL study population, significant linkage disequilibrium exists between rs9527 and rs11191527 (r^2 = 0.28; D' = 0.87). Given the correlation between these two variants, this analysis implemented Burgess *et al.*'s Mendelian randomization technique to construct the instrument using principal components.[19]

The Mendelian randomization analysis was repeated for each of the traits within rice and smoking sub-strata three times: once to estimate the effect of % iAs, once for % MMA and once for % DMA. The genotype-arsenic metabolism effect sizes and standard errors used were as listed in Table 1. The genotype-trait estimates were derived from the HCHS/SOL mixed-effect genetic analyses as described above.

Sensitivity to modelling assumptions

Four of the traits were additionally analysed as dichotomous outcomes at clinically relevant thresholds: systolic blood pressure >130 mmHg,[43] diastolic >80 mmHg,[43] fasting glucose >125 mg/dl[44] and HbA1c >6.5%.[44] Additionally, overall evidence of hypertension [systolic >140 mmHg, diastolic >90 mmHg (to be consistent with previous work in HCHS/SOL) or current antihypertensive medication use[45]] and overall evidence of diabetes (fasting glucose >126 mg/dl, HbA1c 6.5% or current diabetes medication use[46]) were also analysed. To evaluate whether the estimates were sensitive to the control for the sampling design, the analyses were repeated twice: removing the fixed-effect covariate of the log of the sampling weights; and on this unweighted model including education and years in the USA as fixed effects as a proxy of the sampling strata. To evaluate whether the results were sensitive to the top-coding, they were repeated on non-top-coded data. To evaluate whether the results were sensitive to the correction for medication use, the analyses were repeated on uncorrected data where medication use was controlled for as a confounder. To evaluate whether the results were sensitive to the cut-off of rice consumption, the analyses were repeated using rice consumption thresholds of 50th, 66th, 75th and 95th percentiles.

Results

Characteristics of the study population are found in Supplementary Table 1, available as Supplementary data at *IJE* online. Results from the Mendelian randomization of arsenic metabolism and hypertensive traits are presented in Table 2. Among never smokers who are high consumers of rice (Table 2, left top), increased % iAs was associated with increased systolic blood pressure [1.96 mmHg/percentage point increase in %i AS; 95% confidence interval (CI): 0.13 to 3.80; $P = 0.034$] and increased diastolic blood pressure (1.85 mm Hg/percentage point increase; 95% CI: 0.60 to 3.10; $P = 0.003$). Increased % MMA was similarly associated with increased diastolic and systolic blood pressure (1.64 mmHg systolic/percentage point; 95% CI: 0.23 to 3.05; $P = 0.021$; and 1.33 mmHg diastolic/percentage point increase; 95% CI: 0.39 to 2.26; $P = 0.005$). The marker of efficient arsenic metabolism, increased % DMA, was inversely associated with both systolic and diastolic blood pressure (−0.92 mmHg systolic/percentage point increase in % DMA; 95% CI: −1.73 to −0.11; $P = 0.025$; and -0.79 mmHg diastolic/percentage point increase; 95% CI: −1.33 to −0.25; $P = 0.004$). For pulse pressure, the standard errors were consistent with the null hypothesis for all three arsenic metabolites. Among ever smokers (Table 2, right top), the point estimates of the association were smaller and the confidence intervals widened to include zero for all hypertensive traits. Among intermediate- and low-consumers of rice, there was no clear pattern

Table 2. Mendelian randomization estimates for the associations between three measures of arsenic metabolism efficiency and hypertensive traits

	Never smokers		Ever smokers	
	($n = 7535$)		($n = 5074$)	
Metabolite	Beta (95% CI)	*P*-value	Beta (95% CI)	*P*-value
High consumers of rice ($n = 1261$)				
	***n* = 660**		***n* = 601**	
Systolic blood pressure				
% iAs	1.963 (0.130 to 3.796)	0.034	0.232 (−2.133 to 2.596)	0.846
% MMA	1.638 (0.227 to 3.049)	0.021	0.144 (−1.475 to 1.763)	0.860
% DMA	−0.916 (−1.725 to -0.108)	0.025	−0.087 (−1.056 to 0.881)	0.859
Diastolic blood pressure				
% iAs	1.849 (0.603 to 3.095)	0.003	0.180 (−1.260 to 1.620)	0.805
% MMA	1.326 (0.391 to 2.261)	0.005	0.187 (−0.808 to 1.182)	0.710
% DMA	−0.787 (−1.329 to -0.246)	0.004	−0.099 (−0.693 to 0.494)	0.741
Pulse pressure				
% iAs	0.496 (−0.813 to 1.805)	0.453	0.037 (−1.584 to 1.658)	0.964
% MMA	0.572 (−0.423 to 1.568)	0.255	−0.077 (−1.196 to 1.041)	0.891
% DMA	−0.288 (−0.861 to 0.286)	0.320	0.025 (−0.642 to 0.692)	0.941
Low/intermediate consumers of rice ($n = 11\,348$)				
	***n* = 6875**		***n* = 4473**	
Systolic blood pressure				
% iAs	−0.002 (−0.684 to 0.680)	0.996	−0.199 (−1.037 to 0.639)	0.637
% MMA	0.064 (−0.435 to 0.563)	0.800	−0.031 (−0.643 to 0.581)	0.920
% DMA	−0.020 (−0.312 to 0.272)	0.892	0.046 (−0.313 to 0.404)	0.801
Diastolic blood pressure				
% iAs	−0.265 (−0.654 to 0.124)	0.178	−0.140 (−0.631 to 0.351)	0.573
% MMA	−0.161 (−0.447 to 0.125)	0.266	−0.012 (−0.370 to 0.346)	0.947
% DMA	0.101 (−0.066 to 0.267)	0.232	0.027 (−0.182 to 0.237)	0.796
Pulse pressure				
% iAs	0.299 (−0.179 to 0.778)	0.216	−0.063 (−0.650 to 0.525)	0.833
% MMA	0.240 (−0.110 to 0.590)	0.174	−0.022 (−0.451 to 0.407)	0.918
% DMA	−0.132 (−0.337 to 0.072)	0.201	0.020 (−0.231 to 0.271)	0.874

Low/intermediate consumers of rice are those below the 90th percentile of consumption for grains. Systolic blood pressure, diastolic blood pressure and pulse pressure are measured in mmHg. The reported coefficients are interpreted as the expected increase in the trait for a one percentage point increase in the arsenic metabolite.

% iAs, percentage of inorganic arsenic; % MMA, percentage of monomethylarsenate; % DMA, percentage of dimethylarsinate.

between any of the three arsenic metabolites and hypertension for all traits (Table 2, bottom). For the diabetes-related traits (Table 3), we found no evidence of an association between genetically influenced arsenic metabolism efficiency, as the magnitude of the estimated effect was small and the confidence intervals wide. These null results were observed in both high- and low-consumers of rice and every smoking status.

Sensitivity analyses

For the dichotomous hypertensive traits, the directions of the effects for high consumers of rice were consistent with the continuous analyses (Supplementary Table 2, available as Supplementary data at *IJE* online), although the confidence intervals of the estimates were consistent with null association (with the exception of high diastolic blood pressure). For the dichotomous diabetes traits, there continued to be no evidence of an association (Supplementary Table 3, available as Supplementary data at *IJE* online).

The results were not sensitive to the top-coding of outliers in the diabetes-related traits, normality transformations, correction for medication use or methodology to control for study structure. Altering the threshold of 'high' rice consumption produced substantively similar results; however, as the threshold increased, the point estimates increased in magnitude and the smaller sample size produced estimates that were less precise (tables available upon request).

Table 3. Mendelian randomization estimates for the associations between three measures of arsenic metabolism efficiency and diabetic traits

	Never smokers ($n = 7541$)		Ever smokers ($n = 5079$)	
Metabolite	Beta (95% CI)	*P*-value	Beta (95% CI)	*P*-value
High consumers of rice (n = 1263)				
	n = 662		**n = 601**	
Fasting glucose				
% iAs	−0.768 (−3.960 to 2.424)	0.634	−1.259 (−5.806 to 3.287)	0.583
% MMA	−0.584 (−3.111 to 1.942)	0.647	−1.032 (−3.999 to 1.935)	0.491
% DMA	0.332 (−1.098 to 1.762)	0.646	0.590 (−1.212 to 2.392)	0.517
HbA1c				
% iAs	−0.018 (−0.169 to 0.132)	0.812	−0.069 (−0.245 to 0.106)	0.435
% MMA	−0.033 (−0.146 to 0.080)	0.561	−0.067 (−0.189 to 0.055)	0.277
% DMA	0.014 (−0.051 to 0.079)	0.676	0.036 (−0.036 to 0.109)	0.322
ln (HOMA-IR)				
% iAs	0.042 (−0.046 to 0.130)	0.346	0.026 (−0.080 to 0.133)	0.624
% MMA	0.032 (−0.034 to 0.097)	0.344	0.011 (−0.063 to 0.085)	0.766
% DMA	−0.018 (−0.057 to 0.020)	0.343	−0.007 (−0.051 to 0.037)	0.743
ln (HOMA-B)				
% iAs	0.047 (−0.035 to 0.128)	0.255	0.080 (−0.014 to 0.173)	0.091
% MMA	0.033 (−0.029 to 0.095)	0.294	0.054 (−0.011 to 0.119)	0.102
% DMA	−0.019 (−0.055 to 0.016)	0.279	−0.032 (−0.071 to 0.007)	0.100
ln (fasting insulin)				
% iAs	0.039 (−0.038 to 0.117)	0.314	0.037 (−0.057 to 0.131)	0.436
% MMA	0.031 (−0.027 to 0.090)	0.283	0.020 (−0.045 to 0.085)	0.541
% DMA	−0.018 (−0.051 to 0.016)	0.296	−0.013 (−0.051 to 0.026)	0.525
ln (triglycerides)				
% iAs	0.074 (0.008 to 0.140)	0.026	0.014 (−0.059 to 0.088)	0.699
% MMA	0.039 (−0.010 to 0.088)	0.117	0.006 (−0.045 to 0.057)	0.809
% DMA	−0.027 (−0.055 to 0.002)	0.066	−0.004 (−0.034 to 0.026)	0.794
ln (McAuley)				
% iAs	−0.034 (−0.069 to 0.002)	0.059	−0.012 (−0.054 to 0.029)	0.557
Low/intermediate consumers of rice (n = 11 358)				
	n = 6873		**n = 4473**	
Fasting glucose				
% iAs	0.497 (−0.851 to 1.845)	0.465	0.200 (−1.745 to 2.144)	0.839
% MMA	0.406 (−0.588 to 1.400)	0.419	0.144 (−1.284 to 1.571)	0.842
% DMA	−0.227 (−0.807 to 0.352)	0.437	−0.077 (−0.911 to 0.757)	0.854
HbA1c				
% iAs	0.020 (−0.033 to 0.073)	0.456	−0.004 (−0.077 to 0.069)	0.913
% MMA	0.014 (−0.025 to 0.053)	0.478	−0.004 (−0.058 to 0.049)	0.881
% DMA	−0.008 (−0.031 to 0.015)	0.478	0.002 (−0.029 to 0.033)	0.896
ln (HOMA-IR)				
% iAs	−0.016 (−0.043 to 0.012)	0.258	−0.021 (−0.059 to 0.017)	0.270
% MMA	−0.010 (−0.030 to 0.010)	0.312	−0.011 (−0.039 to 0.016)	0.426
% DMA	0.006 (−0.005 to 0.018)	0.275	0.008 (−0.009 to 0.024)	0.355
ln (HOMA-B)				
% iAs	−0.028 (−0.054 to -0.002)	0.033	−0.008 (−0.044 to 0.027)	0.647
% MMA	−0.020 (−0.039 to -0.001)	0.040	−0.002 (−0.028 to 0.024)	0.877
% DMA	0.012 (0.001 to 0.023)	0.036	0.002 (−0.013 to 0.017)	0.793
ln (fasting insulin)				
% iAs	−0.019 (−0.043 to 0.004)	0.105	−0.021 (−0.053 to 0.012)	0.211
% MMA	−0.014 (−0.031 to 0.004)	0.128	−0.011 (−0.035 to 0.012)	0.346
% DMA	0.008 (−0.002 to 0.018)	0.111	0.008 (−0.006 to 0.022)	0.284

(Continued)

Table 3. Continued

	Never smokers ($n = 7541$)		Ever smokers ($n = 5079$)	
Metabolite	Beta (95% CI)	*P*-value	Beta (95% CI)	*P*-value
ln (triglycerides)				
% iAs	−0.009 (−0.028 to 0.010)	0.374	−0.010 (−0.034 to 0.014)	0.406
% MMA	−0.008 (−0.022 to 0.006)	0.277	−0.004 (−0.021 to 0.014)	0.665
% DMA	0.004 (−0.004 to 0.012)	0.314	0.003 (−0.007 to 0.013)	0.533
ln (McAuley)				
% iAs	0.008 (−0.002 to 0.019)	0.118	0.009 (−0.005 to 0.022)	0.189
% MMA	0.006 (−0.001 to 0.014)	0.099	0.005 (−0.005 to 0.014)	0.366
% DMA	−0.004 (−0.008 to 0.001)	0.102	−0.003 (−0.009 to 0.003)	0.276

Low/intermediate consumers of rice are those below the 90th percentile of consumption for grains. Fasting glucose was measured in units of mg/dL; HbA1c is percentage of glycated haemoglobin; fasting insulin is in units of the natural log of mU/L; HOMA-B is in units of the natural log of (mU/L)/(mg/dL); HOMA-IR is in units of the natural log of (mg/dL)·(mU/L); ln (McAuley) index is calculated as 2.63–0.28 ln (fasting insulin)-0.31 ln (fasting triglycerides); triglycerides were measured in units of mg/dl. The reported coefficients are interpreted as the expected increase in the trait for a one percentage point increase in the arsenic metabolite.

% iAs, percent of inorganic arsenic; % MMA, percentage of monomethylarsenate; % DMA, percentage of dimethylarsinate.

Discussion

We find that poor arsenic metabolism efficiency is associated with higher systolic and diastolic blood pressure, and that these effects are seen at levels of arsenic exposure that are present in the Hispanic/Latino community through diet. This association is strongest among never smokers, where each percentage increase in % iAs was associated with approximately 2 mmHg higher systolic and diastolic blood pressure; increases of % MMA were associated with approximately 1.5 mmHg higher blood pressure. Given that the risk alleles were each associated with an increase in % iAs of approximately 2% age points (Table 1), this suggests that arsenic metabolism efficiency may be responsible for clinically meaningful variability in blood pressure among high rice consumers.

Our Mendelian randomization approach uses a novel methodology to complement the existing literature, to suggest that dietary sources of arsenic can provide a level of exposure that can influence blood pressure.[47–49] Given that the HCHS/SOL participants live in urban areas with regulated public water systems that show no evidence of elevated arsenic,[50–53] this suggests that at levels of arsenic exposure that can be acquired through diet, inter-individual differences in the ability to metabolize arsenic can influence arsenic-related health outcomes. The null results in the intermediate- and low-consumers of rice served as a negative control: those who did not consume enough rice to elevate their arsenic exposure did not have their blood pressure affected by their ability to metabolize arsenic.

The results were specific to smoking status. In ever smokers, the effect size was attenuated, and the confidence intervals of the effect size were consistent with no association. This suggests that in smokers, the harms of poor arsenic methylation capacity are less apparent than in people who have never smoked. An assessment of the participants of the UK Biobank (UKBB) found an association between the *AS3MT* SNVs in our instrument and smoking,[54] raising a concern about pleiotropy, but we suggest that pleiotropy through smoking status is unlikely to explain this difference. Population-level analyses have not found a consistent effect of smoking on blood pressure,[31–34] the SNV-smoking effect sizes reported in the UKBB are small in magnitude and inconsistent in direction and there is no association between the *AS3MT* SNVs and smoking in HCHS/SOL [$P > 0.25$ for both, with odds ratios (ORs) close to 1]. Therefore, we suggest that this difference in effect may be evidence of an interaction between smoking and arsenic metabolism, and that additional investigations into this mechanism are warranted. Although previous work has found that the health effects of the absolute level of arsenic exposure differ by smoking status,[55–57] few have examined evidence for an interaction between arsenic methylation and smoking.

The analysis did not provide evidence of association with any of the seven diabetes-related traits tested. Whereas future research that directly measures arsenic exposure may be able to provide more precise estimates, this is consistent with other work that has not found a strong link between low-and intermediate-levels of arsenic exposure and diabetes.[47,58,59]

Although there are several methods for incorporating multiple SNVs into Mendelian randomization analyses,

including polygenic risk scores,[60] MR-Eggers[61] and inverse variant weights,[62] this analysis used the Burgess methodology[19] that transforms the SNVs into their principal components. Given the strong linkage disequilibrium in the *AS3MT* region,[63] the Burgess principal components analysis (PCA) method most comprehensively removes the possibility of inflating type I error due to correlation between rs9527 and rs11191527.

The results of this study could be strengthened if additional data were available. A direct measure of arsenic exposure could have classified iAs exposure more precisely than rice consumption groups. However, given the low water arsenic exposure of the HCHS/SOL participants, it is likely that dietary arsenic was a main source of exposure. Any misclassification that resulted from non-dietary routes of arsenic exposure or imperfect food recall would likely be non-differential, and therefore make the estimates less precise but not confounded. Additionally, it would have been preferable to have ascertained the SNV-arsenic metabolism association in a study population with Hispanic/Latino ancestry, rather than the South Asian population that was represented in the published literature. However, the association between variants in the *AS3MT* region and arsenic metabolites has been observed in multiple genetic backgrounds.[64–66] This suggests that although the SNVs in Table 1 may tag the truly causal SNV less efficiently in the HCHS/SOL population, this would only bias the results towards the null. Fine-mapping of the *AS3MT* and *FTCD* regions will provide additional insight into the causal SNVs, which would improve the precision of these estimates.

Mendelian randomization assumptions

The interpretation of these results depends on the standard Mendelian randomization assumptions:[67] (i) the SNVs from the literature predict % iAs, % MMA and % DMA; (ii) the SNVs are not confounded; and (iii) the SNVs only affect the hypertensiion- and diabetes-related traits through their effects on arsenic metabolism.

Although the first assumption cannot be tested directly since urinary arsenic metabolites were not measured in HCHS/SOL, its plausibility is supported first by the established biological pathways that connect the three SNVs to arsenic metabolism efficiency; rs9527 and rs11191527 are located near *AS3MT*, a gene that encodes arsenite methyltransferase, an enzyme involved in the arsenic metabolism process;[9,68] and rs61735836 produces a missense mutation in *FTCD*, which encodes formimidoyltransferase cyclodeaminase, an enzyme critical to arsenic metabolism.[69] Additionally, this analysis only included SNVs whose associations with % iAs, % MMA and % DMA reached a stringent threshold in the original studies.[62,70]

The strongest threat to the second assumption often comes in the form of uncontrolled population stratification.[71] In this analysis, the SNV-trait relationships were estimated within HCHS/SOL using a well-developed algorithm that controls for cryptic relatedness, sample clustering, ancestral background groups and principal components.[35,42] This framework has undergone extensive validation in HCHS/SOL and other admixed populations, and has been shown to be able to provide estimates with no overall inflation of type I error.[72]

The third assumption requires that there are no other non-arsenic metabolism-related genetic influences of blood pressure through any of the SNVs used in the instrument. Whereas this assumption is not fully testable, we undertook two robustness checks. First, the NHGRI-EBI GWAS catalogue[73] was searched to verify that no variants in high linkage disequilibrium ($r^2 > 0.3$) with any of the SNVs in the instrument are known to be associated with cardiovascular or diabetes-related traits, or with carbohydrate metabolism. Second, a final sensitivity analysis was performed that restricted the instrument to SNVs where the SNV-arsenic metabolism relationship from the literature was roughly proportional to the SNV-outcome relationship in HCHS/SOL, as this proportionality can be an indicator that the third assumption holds.[74] Of the three SNVs included in the instrument, the per-allele effect of one of them (rs11191527) was in the opposite direction expected for the hypertensive traits and was therefore excluded from the instrument in the final sensitivity analysis. The output of this sensitivity analysis produced substantively similar results to the three-SNV instrument, but the magnitudes of the associations were attenuated and the confidence intervals widened (Supplementary Table 4, available as Supplementary data at *IJE* online).

In conclusion, this study suggests that high rice consumption confers a risk of arsenic-related increase in blood pressure, and that this increased risk is felt most acutely in non-smokers who inefficiently metabolize arsenic. This finding highlights a previously unappreciated risk factor for hypertension in the Hispanic/Latino community. These findings directly suggest diet-related mitigation efforts that could help to reduce the burden of hypertension, and future research that could more fully elucidate the magnitude of this risk.

Supplementary Data

Supplementary data are available at *IJE* online.

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