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Arsenic and gallbladder cancer risk: Mendelian randomization analysis of European prospective data

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Dear editor,

An inverse association between arsenic in serum and the risk of gallbladder cancer (GBC) was recently reported in a cross-sectional study conducted by Lee *et al.* in Shanghai, China.[1] This result was surprising, because arsenic has been classified as a human carcinogen and arsenic-contaminated water has recently been associated with increased risk of GBC.[2,3] Motivated by this unexpected finding, we applied Mendelian randomization (MR) to assess the causal effect of arsenic on GBC risk.

Once arsenic in drinking water and food is absorbed into the bloodstream, inorganic arsenic (iAs) is methylated to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) to facilitate excretion in urine.[4] Lee *et al.* used inductively coupled plasma mass spectrometry to measure total arsenic in serum but made no distinction among arsenic species. The authors categorized the arsenic levels based on tertiles (Ts) because they noticed a nonlinear relationship between total arsenic and GBC risk. The reported odds ratios (OR) adjusted for age, sex, body mass index, cigarette smoking, alcohol consumption and levels of triglycerides and cholesterol were OR = 0.38 for T2 *versus* T1 and OR = 0.20 for T3 *versus* T1 (p trend <0.001). In the discussion of their findings, Lee *et al.* hypothesize that the inverse association could be attributed to decreased seafood intake by GBC patients; however, information on the amount, frequency and types of seafood consumed by study participants was not available. The authors also postulate that the inverse association could be due to impaired viability and apoptosis of cancer cells after arsenic exposure, but the case–control study design did not permit assessment of whether arsenic exposure preceded GBC development. In addition to the impossibility of (*i*) distinguishing between arsenic species and (*ii*) ruling out reverse causality (i.e., GBC causes decreased arsenic levels rather than *vice versa*), (*iii*) potential confounding was another limitation of the study by Lee *et al.* For example, diabetes has been associated with both arsenic exposure and GBC, and these associations could negatively distort the observed relationship between increased arsenic levels in serum and decreased GBC risk.[5,6]

The capacity to metabolize arsenic shows considerable interindividual variation, depending partly on the genetic variants inherited by an individual. The relative abundance of arsenic species in urine reflects the individual capacity for arsenic elimination: increased iAs% and MMA%, and decreased DMA% are indicative of poor metabolizing

efficiency, which in turn results in a high biologically effective dose of arsenic exposure.[4] MR permits assessment of the causal effect of a risk factor (here, the percentages of arsenic species) on a particular phenotype (here, GBC development) using genetic variants as instrumental variables.[7] MR makes it possible to rule out the potential effects of reverse causality and confounding. We applied two-sample MR to examine the causal effects of iAs%, MMA% and DMA% on GBC risk. The methodology proposed by Burgess et al., which has been previously applied to other MR studies of arsenic, was used to integrate genotype-arsenic metabolism summary statistics from the literature with genotype-GBC risk summary statistics adjusted for age, gender and the first five genetic principal components based on a collaborative European study set up with the participation of the European Prospective Investigation into Cancer and Nutrition Cohort, the Nord-Trøndelag Health Study, the ESTHER Study, the Swedish Twin Registry, the National FINRISK Study, the Study of Health in Pomerania, the Estonian Genome Project and Lifelines.[8] Ethics approval was obtained for all studies and informed consent was provided by all participants. Statistics on the association between the percentages of arsenic species and the instrumental variables rs9527 and rs11191527 near *AS3MT*, and rs61735836 in exon 3 of *FTCD* were retrieved from two publications.[4,9] Additive mixed linear regression models were used for association testing in two study populations comprising 2,060 (*AS3MT*) and 1,660 (*FTCD*) arsenic-exposed Bangladeshi individuals. The variance in relative abundance of arsenic species explained by the considered genetic variants (for example, ~10% for DMA%) and the available sample size (103 prospective cases and 168 controls) translated into a detectable OR of around 0.39 per standard deviation (SD; type I error rate of 5%).[10]

In agreement with the surprising results of Lee *et al.*, we found evidence for a protective effect of iAs% on GBC risk (OR = 0.80, p = 0.03, Fig. 1). Adding plausibility to this finding, poor metabolizing capacity, marked by MMA%, also showed a protective effect (OR = 0.85, p = 0.08) and DMA%, a marker of efficient arsenic metabolism, showed a deleterious effect on GBC risk (OR = 1.10, p = 0.06). The variants rs9527 (*AS3MT*) and rs61735836 (*FTCD*) showed consistent ORs (Fig. 1). The variant rs11191527 near *AS3MT* gene showed discrepant results and broader 95% confidence intervals for iAs% and MMA%.

The present MR study has some limitations. GBC is relatively rare in Europe, and the investigated collective was small compared to traditional MR studies. The measured variation of arsenic species in the study by Pierce *et al.*—SD = 6.4 for iAs%, SD = 5.1 for MMA% and SD = 8.5 for DMA% (personal communication)—probably results in lower detectable causal ORs for DMA% than for MMA% or iAs%.[4] The genetic variants used for MR may not be the best predictors of the individual capacity to metabolize and eliminate arsenic for Europeans: the utilized summary statistics on genotype-arsenic metabolism relied on a study of arsenic exposure in Bangladesh. Differences in allele frequency, linkage disequilibrium patterns and arsenic exposure across populations could translate into alternative, stronger predictors of arsenic elimination efficiency for Europeans. For example, the minor allele frequency of rs9527 near *AS3MT* is 8% in Bangladeshis compared to 25% in Europeans (ensembl.org). The r^2 between *AS3MT* variants rs9527 and rs11191527 is 0.04 for Bangladeshis and 0.36 for Europeans, and Pierce *et al.* explicitly state that rs11191527 may not be a strong instrumental variable for DMA% in populations with low arsenic exposure.[4] In spite of these limitations, we consider that our MR results may contribute to the meager literature on GBC and hopefully motivate future collaborative research to raise the available sample sizes.

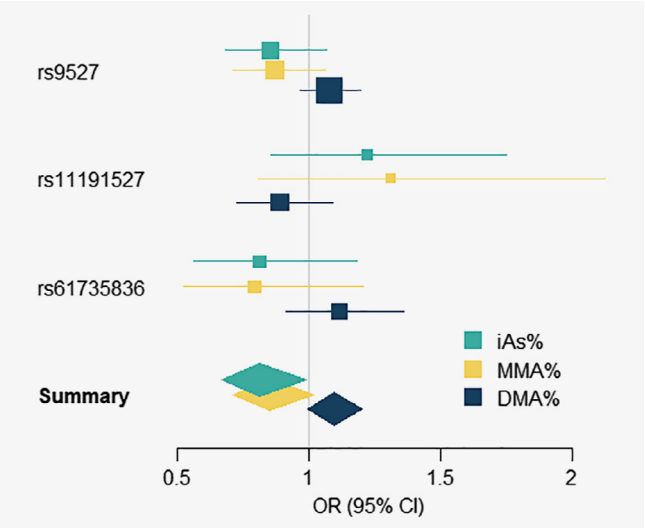

Figure 1. Odds ratios (OR) and corresponding 95% confidence interval (95% CI) for the association between *AS3MT* variants rs9527 and rs11191527, and variant rs61735836 in *FTCD* as instrumental variables of the individual capacity to metabolize arsenic and GBC risk. The summary OR was calculated using the methodology proposed by Burgess *et al.* for linked genetic variants.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Conflict of interest

None declared.

Disclosure

All authors have nothing to disclose. The authors from the International Agency for Research on Cancer/World Health Organization alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

References

1. Lee MH, Gao YT, Huang YH, et al. A metallomic approach to assess associations of serum metal levels with gallstones and gallbladder cancer. *Hepatology* 2020. https://doi.org/10.1002/hep.30861. [Epub ahead of print].
2. Ganesan N, Bambino K, Boffetta P, et al. Exploring the potential carcinogenic role of arsenic in gallbladder cancer. *Eur J Cancer Prev* 2020. https://doi.org/10.1097/CEJ.0000000000000521. [Epub ahead of print].
3. Straif K, Benbrahim-Tallaa L, Baan R, et al. A review of human carcinogens—part C: metals, arsenic, dusts, and fibres. *Lancet Oncol* 2009;10:453–4.
4. Pierce BL, Tong L, Argos M, et al. Arsenic metabolism efficiency has a causal role in arsenic toxicity: Mendelian randomization and gene-environment interaction. *Int J Epidemiol* 2013;42:1862–71.
5. Scannell Bryan M, Sofer T, Mossavar-Rahmani Y, et al. 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. *Int J Epidemiol* 2019;48:876–86.
6. Sung TC, Huang JW, Guo HR. Association between arsenic exposure and diabetes: a meta-analysis. *Biomed Res Int* 2015;2015:368087.
7. Lawlor DA, Harbord RM, Sterne JA, et al. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med* 2008;27:1133–63.
8. Burgess S, Zuber V, Valdes-Marquez E, et al. Mendelian randomization with fine-mapped genetic data: choosing from large numbers of correlated instrumental variables. *Genet Epidemiol* 2017;41:714–25.
9. Pierce BL, Tong L, Dean S, et al. A missense variant in FTCD is associated with arsenic metabolism and toxicity phenotypes in Bangladesh. *PLoS Genet* 2019;15:e1007984.
10. Brion MJ, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. *Int J Epidemiol* 2013;42:1497–501.

Key words: arsenic, gallbladder cancer, Mendelian randomization

Abbreviations: AS3MT: arsenite methyltransferase; DMA: dimethylarsinic acid; ESTHER: Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung; FTCD: formimidoyltransferase cyclodeaminase; GBC: gallbladder cancer; iAs: inorganic arsenic; MMA: monomethylarsonic acid; MR: Mendelian randomization; OR: odds ratio; SD: standard deviation; T: tertile

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