ENVIRONMENTAL EPIGENETICS

Environmental Epigenetics, 2022, **8(1)**, 1–6

DOI: https://doi.org/10.1093/eep/dvac014
Advance Access Publication 1 June 2022
Technical Briefs

The impact of prenatal and early-life arsenic exposure on epigenetic age acceleration among adults in Northern Chile

Anne K. Bozack[1], Philippe Boileau[2], Alan E. Hubbard[2], Fenna C. M. Sillé[3], Catterina Ferreccio[4], Craig M. Steinmaus[1], Martyn T. Smith[1] and Andres Cardenas[1,*]

[1]Division of Environmental Health Sciences, School of Public Health, University of California, 2121 Berkeley Way, Room 5302, Berkeley, CA 94720, USA, [2]Graduate Group in Biostatistics, University of California, 2121 Berkeley Way, Room 5302, Berkeley, CA 94720, USA, [3]Department of Environmental Health and Engineering, The Johns Hopkins University Bloomberg School of Public Health, 615 N. Wolfe Street, Room E7527, Baltimore, MD 21205, USA, [4]Advanced Center for Chronic Diseases (ACCDiS), School of Medicine, Pontificia Universidad Católica de Chile, Sergio Livingstone 1007, Independencia, Santiago, Chile
*Correspondence address. Division of Environmental Health Sciences, School of Public Health, University of California, 2121 Berkeley Way, Room 5302, Berkeley, CA 94720, USA. Tel: +510-643-0965; E-mail: andres.cardenas@berkeley.edu

Abstract

Exposure to arsenic affects millions of people globally. Changes in the epigenome may be involved in pathways linking arsenic to health or serve as biomarkers of exposure. This study investigated associations between prenatal and early-life arsenic exposure and epigenetic age acceleration (EAA) in adults, a biomarker of morbidity and mortality. DNA methylation was measured in peripheral blood mononuclear cells (PBMCs) and buccal cells from 40 adults (median age = 49 years) in Chile with and without high prenatal and early-life arsenic exposure. EAA was calculated using the Horvath, Hannum, PhenoAge, skin and blood, GrimAge, and DNA methylation telomere length clocks. We evaluated associations between arsenic exposure and EAA using robust linear models. Participants classified as with and without arsenic exposure had a median drinking water arsenic concentration at birth of 555 and 2 μg/l, respectively. In PBMCs, adjusting for sex and smoking, exposure was associated with a 6-year PhenoAge acceleration [*B* (95% CI) = 6.01 (2.60, 9.42)]. After adjusting for cell-type composition, we found positive associations with Hannum EAA [*B* (95% CI) = 3.11 (0.13, 6.10)], skin and blood EAA [*B* (95% CI) = 1.77 (0.51, 3.03)], and extrinsic EAA [*B* (95% CI) = 4.90 (1.22, 8.57)]. The association with PhenoAge acceleration in buccal cells was positive but not statistically significant [*B* (95% CI) = 4.88 (–1.60, 11.36)]. Arsenic exposure limited to early-life stages may be associated with biological aging in adulthood. Future research may provide information on how EAA programmed in early life is related to health.

Key words: arsenic; environmental exposure; epigenetic age acceleration; peripheral blood mononuclear cells

Introduction

Arsenic exposure through drinking water is an ongoing global public health concern with >200 million people exposed to levels above the World Health Organization (WHO) guideline of 10 μg/l [1]. Arsenic is a human toxicant and carcinogen and increases the risk of adverse health outcomes, including cardiovascular disease, diabetes, impaired neurodevelopment, and cancer [2]. Elevated disease risk persists decades after exposure has ended or been reduced, suggesting that changes in the epigenome may be involved in biological pathways linking arsenic to health [3]. Arsenic has been associated with changes in global, regional, and locus-specific DNA methylation (DNAm) [4]. However, it is not known if arsenic impacts epigenetic age acceleration (EAA), a biomarker of disease risk and mortality [5].

A unique exposure scenario in the current study area in Northern Chile has provided an opportunity to investigate long latency periods of arsenic-related health conditions. In this study area, drinking water was sourced from an arsenic-contaminated river, exposing residents to high levels of arsenic during the period of 1958–72. Previous studies have found that the risk of incident cancer, including lung and bladder [6, 7], and cancer and non-cancer mortality [8–10] remained elevated after exposure had ended. Risks of mortality from bronchiectasis and bladder, laryngeal, and lung cancers were the highest for exposures during prenatal or early-life periods, suggesting critical windows of susceptibility [10] coinciding with periods of epigenetic programming and the establishment of DNAm signatures [11].

Changes in DNAm have been found to be associated with aging and lifespan. Several epigenetic biomarkers of chronological and biological ages have been developed, including the Horvath multi-tissue estimator [12], the Hannum estimator [13], and the skin and blood clock [14]. To better capture physiological changes resulting in morbidity and mortality, the PhenoAge [15] and GrimAge [16] estimators have incorporated clinical biomarkers of aging into training models. EAA, or the difference between epigenetic age and chronological age, may provide a measure of biological aging

Received: 21 March 2022; **revised:** 2 May 2022; **accepted:** 31 May 2022

of a tissue relative to that expected based on chronological age. As reviewed by Horvath and Raj, EAA has been associated with a broad range of age-related health conditions, including cancer, cardiovascular disease, and frailty, and other aging biomarkers such as C-reactive protein and triglycerides [5]. EAA calculated from PhenoAge has been shown to be a better predictor of the risk of coronary heart disease and all-cause mortality compared to the Horvath and Hannum estimators [15] and a better predictor of the number of comorbidities compared to the Horvath, Hannum, and GrimAge estimators [16]. However, GrimAge slightly outperforms PhenoAge in the prediction of time to death, time to coronary heart disease, and time to cancer [16]. DNAm-based biomarkers of aging can also be distinguished by their relationship to cell-type composition. The proportion of blood cell types are known to change with age. Whereas the Horvath multi-tissue estimator is largely independent of variations in cell types and can be applied to measuring intrinsic aging, biomarkers including the Hannum and PhenoAge estimators capture changes in age-related cell-type proportions, better reflecting extrinsic aging [5].

Increased rate of epigenetic aging may be related to biological pathways linking adverse environmental exposures to health outcomes or may serve as a biomarker of environmental risk factors; however, few studies have investigated associations between environmental exposures and EAA, as reviewed by Ryan *et al.* [17]. In the current study, we leveraged data from unexposed adults and adults with high arsenic exposure confined to the prenatal and early-life periods to assess the extent to which historic high arsenic exposure impacts EAA. DNAm was measured in peripheral blood mononuclear cells (PBMCs) and buccal cells collected in adulthood, and EAA was calculated using six epigenetic biomarkers developed to estimate chronological age, morbidity and mortality, and telomere length (TL). We hypothesized that prenatal and early-life arsenic exposure would be associated with adult EAA, particularly among measures related to disease phenotypes and morbidity.

Methods

The study population and DNAm measurements have previously been described [18]. The study area is located in Region II of Chile, which includes the city of Antofagasta. Adults were recruited at the Antofagasta Hospital or the University of Antofagasta, Chile, using convenience sampling. Between 1958 and 1972, drinking water arsenic was elevated in Region II (e.g. 287 and 860 μg/l in Calama and Antofagasta, respectively), after which concentrations were reduced with arsenic removal plants [7]. Participants provided informed consent, and protocols were approved by the Pontificia Universidad Católica de Chile and the University of California, Berkeley Institutional Review Boards.

Participants born in Region II between 1958 and 1972 were classified as having prenatal or early-life exposure; all were exposed prenatally, and a large proportion was exposed from birth to ∼14 years of age. Participants who were born outside of Region II and moved to Antofagasta after the period of high exposure were classified as unexposed. For all participants, water arsenic concentrations at birth from major municipal sources were also recorded. It should be noted that although the majority of participants in the unexposed group had detectable water arsenic concentrations at birth, only two had concentrations of 15 μg/l at birth, exceeding the current WHO standard of 10 μg/l but substantially lower than the standard of 50 μg/l that was in effect in Chile until 2005 [19]. Yearly municipal water arsenic levels were used to calculate the average lifetime water arsenic concentrations from the age of 20 years until sample collection as well as birth water arsenic levels [20]. Smoking status was classified as ever or never smoker based on self-report.

In 2013, PBMCs and buccal cell samples were collected from adults and stored at −80°C. Samples were transported to the University of California, Berkeley, where DNA was extracted and quantified. DNAm was measured using the Infinium Human MethylationEPIC BeadChip (Illumina, San Diego, CA) at the California Institute for Quantitative Biosciences.

Data were processed with standard quality control procedures [18] and normalized using *funnorm* [21]. One buccal cell sample was removed due to poor quality; one PBMC and one buccal cell sample were removed due to a sex mismatch. In PBMCs, cell-type proportions ($CD8^+$ T cells, $CD4^+$ T cells, natural killer cells, B cells, monocytes, and neutrophils) were estimated using the Houseman regression calibration method [22] implemented with the *estimateCellCounts* function in the R package *minfi* [23]. The following DNAm aging biomarkers were calculated using Horvath's new online calculator [12] (http://dnamage.genetics.ucla.edu/): Horvath [12], Hannum [13], skin and blood [14], PhenoAge [15], and GrimAge clocks [16]. DNAm telomere length (DNAmTL) was also estimated [24]. These biomarkers are summarized in Supplementary Table S1, including health outcomes and tissues used in training. The predictive accuracy of the biomarkers in PBMCs and buccal cells was assessed by calculating Pearson's correlations and median absolute error between estimated values and chronological age.

For each of the biomarkers, EAA (i.e. the difference between epigenetic age and chronological age in years or an age-adjusted estimate of TL in kb) was computed by Horvath's online calculator as the residuals of regressing epigenetic age on chronological age. Intrinsic EAA (IEAA) and extrinsic EAA (EEAA) were also calculated for PBMCs. IEAA is the residual of Horvath's DNAm age on chronological age adjusting for blood cell-type estimates and is independent of age-related changes in cellular heterogeneity. EEAA is calculated from a combined measure of the Hannum DNAm age estimate and blood cell types known to change with age (i.e. enhanced Hannum DNAm age), which is then regressed on chronological age.

We evaluated associations between arsenic exposure (exposed prenatally and in early life vs. not exposed) and EAA in years, or the residuals of kb for DNAmTL, using robust linear regression. Robust linear models were implemented using the *rlm* function in the R *MASS* package [25] using the M estimator. Confidence intervals and *P*-values were calculated using the *lmtest* [25] and *sandwich* [26, 27] R packages with the vcovHC covariance matrix estimation function with White's estimator [28]. We observed the greatest correlations between DNAm aging biomarkers and chronological age in PBMCs, and therefore our primary analyses focused on these samples. Associations between arsenic exposure and EAA calculated in buccal cells were investigated in secondary analyses. We calculated the EAA effect size necessary to detect a significant association with arsenic exposure using the *pwr* R package [29]. With a sample size of 39, at $\alpha = 0.05$, we have 80% power to detect a moderate effect size of $f^2 = 0.27$.

Considering extrinsic measures of EAA capture age-related changes in cell-type proportions, primary analyses were adjusted for sex and smoking status. Sensitivity analyses were conducted with additional adjustments for estimated cell-type proportions in PBMCs. We also performed sensitivity analyses using $\log_2$-transformed estimates of water arsenic concentrations at birth as the exposure and adjusting models for the average lifetime arsenic concentrations between the age of 20 years and sample collection.

Table 1: Participant characteristics

	Prenatal and early-life arsenic exposure ($N = 20$)[a]		Unexposed ($N = 19$)	
	N or Median	% or (IQR)	*N* or Median	% or (IQR)
Female	10	50%	9	47%
Age	49	(44, 53)	49	(46, 53)
Smoking	15	75%	8	42.1%
Drinking water arsenic concentration at birth (μg/l)	555	(130, 680)	2	(1, 6)
Average drinking water arsenic concentration (μg/l), from the age of 20 years until sample collection	28.5	(24.8, 35.1)	15.7	(4.4, 27.0)

[a]$N = 19$ participants with available buccal cell data.

Statistical significance was evaluated using a nominal *P*-value of <0.05. Analyses were conducted using R 4.1.2 [30].

Results

Participant characteristics

PBMC DNAm data were available for 39 participants (20 with prenatal or early-life exposure and 19 unexposed); buccal cell data were available for 38 participants (19 exposed and 19 unexposed). Nineteen participants were female, and the median age was 49 years at sample collection (Table 1). Among participants with prenatal and early-life arsenic exposure, the median (range) drinking water arsenic concentration at birth was 555 (110–860) μg/l, whereas among unexposed participants the median (range) was 2 (0–15) μg/l. The average drinking water arsenic concentration from the age of 20 years until sample collection was similar between the two groups [exposed median (range) = 29 (17–41) μg/l; unexposed median (range) = 16 (0–35) μg/l].

Epigenetic age

In PBMCs, chronological age was positively correlated with DNAm aging biomarkers ($r_{Pearson}$ range: 0.62–0.86; $P < 0.001$) and negatively correlated with DNAmTL ($r_{Pearson} = -0.69$; $P < 0.001$; Fig. 1). In buccal cells, correlations were significant although weaker and imprecise across all DNAm aging biomarkers (DNAm age $r_{Pearson}$ range: 0.39–0.72; $P < 0.05$; DNAmTL $r_{Pearson} = -0.39$; $P = 0.016$).

Arsenic exposure and EAA

In adjusted linear models of EAA in PBMCs, prenatal and early-life arsenic exposure was associated with a 6-year PhenoAge EAA [*B* (95% CI) = 6.01 (2.60, 9.42)] (Fig. 2; Supplementary Table S2). Although associations with all other measures of EAA were not statistically significant, likely influenced by limited statistical power to detect moderate effect sizes, there was a consistently positive direction of the association, with the greatest effect sizes observed for Hannum EAA [*B* (95% CI) = 2.51 (−0.81, 5.83)] and EEAA [*B* (95% CI) = 3.66 (−0.91, 8.23)] (Fig. 2; Supplementary Table S2).

In sensitivity analyses of PBMCs adjusting for estimated cell-type proportions, associations between prenatal and early-life arsenic exposure and PhenoAge EAA were slightly attenuated [*B* (95% CI) = 4.93 (1.88, 7.99)] (Fig. 2; Supplementary Table S3). However, associations with Hannum EAA [*B* (95% CI) = 3.11 (0.13, 6.10)] and EEAA [*B* (95% CI) = 4.90 (1.22, 8.57)] increased and were statistically significant. In addition, we observed a significant positive association with skin and blood EAA after adjusting for cell types [*B* (95% CI) = 1.77 (0.51, 3.03)]. In sensitivity analyses evaluating associations with water arsenic concentrations at birth, PhenoAge acceleration remained significant [*B* (95% CI) = 0.32 (0.003, 0.63) years for each doubling in arsenic concentrations; Supplementary Table S4]. Hunnam EAA and EEAA had positive directions of association but remained imprecise [Hunnam: *B* (95% CI) = 0.22 (−0.04, 0.48); EEAA: *B* (95% CI) = 0.29 (−0.092, 0.67)]. In models adjusting for the average lifetime water arsenic concentration from the age of 20 years to sample collection, the association between exposure status and PhenoAge acceleration slightly increased [*B* (95% CI) = 6.65 (1.91, 11.38)]; however, associations with Hunnam EAA and EEAA were attenuated [Hunnam: *B* (95% CI) = 1.58 (−2.80, 5.96); EEAA: *B* (95% CI) = 2.03 (−3.66, 7.73); Supplementary Table S5].

In analyses of buccal cells, we observed positive but imprecise associations with PhenoAge acceleration [*B* (95% CI) = 4.88 (−1.60, 11.36)], Horvath EAA [*B* (95% CI) = 2.31 (−1.04, 5.65)], and IEAA [*B* (95% CI) = 2.38 (−1.29, 6.06)] (Supplementary Table S6). Associations with other buccal cell EAA biomarkers were null [Hannum: *B* (95% CI) = −1.67 (−6.42, 3.07); skin and blood: *B* (95% CI) = 0.62 (−2.14, 3.39); GrimAge: *B* (95% CI) = −1.69 (−5.45, 2.24); DNAmTL: *B* (95% CI) = 0.18 (−0.18, 0.55); EEAA: *B* (95% CI) = −1.81 (−7.26, 3.64)].

Discussion

These findings are novel highlighting the impact of early-life arsenic exposure on EAA in adults across two tissues. Epigenetic age was significantly correlated with chronological age, although correlations were stronger in PBMCs, as expected since these epigenetic biomarkers were primarily developed using blood samples. We found that adults exposed to high water arsenic concentrations prenatally and in early life had a mean PhenoAge acceleration of 6 years greater than adults who were not exposed. After adjusting for estimated cell-type proportions, we also observed significant positive associations between arsenic exposure and Hannum EAA, skin and blood EAA, and EEAA. No associations between arsenic exposure and EAA measures in buccal cell samples were statistically significant.

Multiple epigenetic biomarkers have been developed to estimate distinct aspects of aging, including chronological age, morbidity, and mortality in specific tissue types. Chronological age estimators include the Horvath multi-tissue predictor, developed using 51 tissues and cell types [12], the Hannum predictor, developed using adult whole blood [13], and the skin and blood clock, developed using eight types of skin- and blood-derived tissues [14]. PhenoAge was developed to predict aging outcomes (e.g. disease count and physical functioning) and mortality using adult blood DNAm and was trained on an estimate of phenotypic age, a measure derived from chronological age and nine clinical markers of aging [15]. GrimAge, a predictor of lifespan, was developed using adult blood DNAm trained on plasma proteins associated with

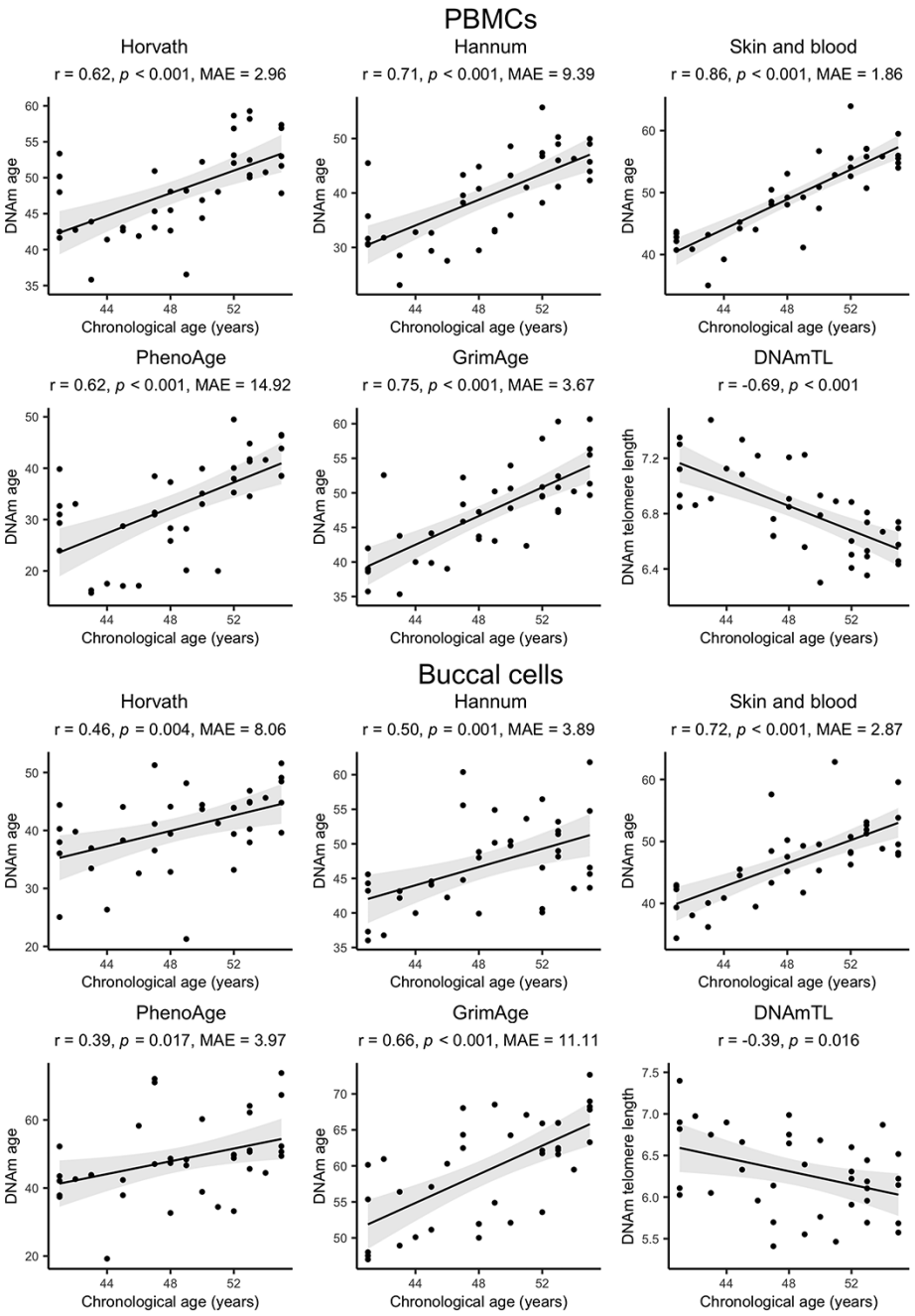

Figure 1: Scatter plots of chronological age and DNAm age. Chronological age (years) is plotted on the x-axis, and DNAm age (years) predicted by the Horvath, Hannum, PhenoAge, skin and blood, and GrimAge clocks or DNAmTL (kb) is plotted on the y-axis. Linear trend lines, 95% CIs, Pearson's correlations, and median absolute error are indicated. MAE = median absolute error

morbidity and mortality and smoking pack-years [16]. Considering that these epigenetic aging biomarkers were primarily derived using blood DNAm, it is not surprising that we observed stronger correlations between epigenetic age and chronological age calculated in PBMCs than buccal cells. Furthermore, the lack of a biomarker developed to estimate epigenetic age in buccal cells likely contributed to imprecise estimates in our analyses.

PhenoAge acceleration measured in PBMCs was most strongly associated with early-life arsenic exposure in our study independent of lifetime exposure and cellular heterogeneity. Clinical biomarkers used in training PhenoAge include alkaline phosphatase and C-reactive protein [15], factors that may also be responsive to arsenic exposure [31, 32]. In a study of serum enzymes associated with liver function among arsenic-exposed Bangladeshi adults, alkaline phosphatase was positively associated with the arsenic levels measured in drinking water, hair, and nails [31]. A separate study of inflammatory markers among Bangladeshi adults found a positive association between water arsenic concentrations and C-reactive protein levels [32]. In enrichment analysis of differentially expressed genes, PhenoAge acceleration has been associated with pro-inflammatory and DNA repair pathways [15]. Furthermore, arsenic exposure and PhenoAge share common health outcomes, including cardiovascular disease and cancer [2, 15]. In particular, arsenic is

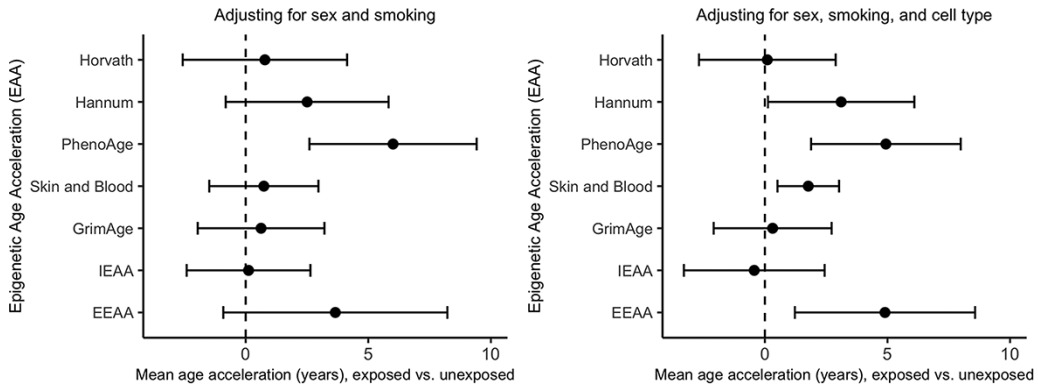

Figure 2: Mean EAA associated with prenatal or early-life arsenic exposure. Mean difference in EAA in years and 95% CIs between participants with and without prenatal or early-life arsenic exposure calculated using linear models adjusted for sex and smoking (left panel) and adjusted for sex, smoking, and estimated cell-type proportions (right panel)

a well-established risk factor for lung cancer among non-smokers [33]; PhenoAge is associated with an increased risk of lung cancer incidence or mortality adjusting for smoking status and smoking pack-years [15]. PhenoAge acceleration has also been associated with smoking [34] and air pollution [35], suggesting that it may capture perturbations in biological processes that link environmental exposures to adverse health outcomes.

Biological mechanisms through which prenatal and early-life environmental exposures affect EAA later in life are not fully understood but may involve changes in inflammatory and immune-related pathways. Our *a priori* hypothesis was that adjustment for cellular heterogeneity would attenuate associations with EAA, particularly extrinsic biomarkers reflective of aging-related changes in cell types. In sensitivity analyses adjusting for cell types, we did observe an attenuation in the association between exposure and PhenoAge EAA from 6.01 to 4.94 years. However, associations with Hannum EAA, skin and blood EAA, and EEAA increased and were statistically significant. Adjusting for cellular proportions may increase the precision of these estimates by controlling for health and environmental factors in adulthood that influence blood cell types. These results suggest that prenatal and early-life arsenic exposure affects pathways involved in aging and mortality independent of aging-related changes in cellular composition.

This study is novel in analyzing associations between drinking water arsenic exposure and EAA. Furthermore, the unique exposure scenario in Northern Chile allowed us to investigate the long-term effects of arsenic on biological aging decades after exposure. Our primary analyses utilized dichotomized exposure data to minimize exposure misclassification; however, sensitivity analyses using prenatal water arsenic concentrations yielded consistent results. PhenoAge acceleration was also significantly associated with prenatal and early-life exposure after adjusting for the average water arsenic concentration in adulthood, providing evidence that observed associations are attributable to exposure during critical periods of development and epigenetic programming.

Our study had several limitations. First, we were limited by a small sample size and reduced power to detect significant associations. Studies with larger samples size are needed to verify if observed associations are due to unmeasured confounding or artifact and if associations with other biomarkers of aging were not statistically significant due to limited power. Secondly, chronological age at sample collection was available by year increments; higher resolution data could increase precision by including a wider range of ages. Our study was also limited to a population in Northern Chile with high early-life exposure, affecting the generalizability of findings. Future studies are needed to fully understand arsenic-associated EAA in diverse populations with exposures at different life stages, particularly during pregnancy and in early life.

Our results provide evidence for environmentally induced EAA, particularly relating to measures of morbidity and mortality, and suggest that arsenic exposure limited to early-life stages is associated with tissue-specific biological aspects of aging measured decades later. Additional research is needed to investigate how arsenic-associated EAA is related to health and if biological aging in mid-life is programmed in early life.

Data availability

The datasets generated and/or analyzed during the current study are not publicly available due to participant confidentiality but are available from the corresponding author on reasonable request and upon Institutional Review Board review. The code used in data processing, analysis, and visualization of results is available at the study's GitHub repository (https://github.com/annebozack/Chile_arsenic_EAA).

Supplementary data

Supplementary data is available at EnvEpig online.

Acknowledgements

We thank our staff, the fieldworkers, and the study participants in Chile for making this work possible. We would also like to acknowledge Dr Jorge Gonzalez, in the Molecular Parasitology Unit, Medical Technology Department, University of Antofagasta, Antofagasta, Chile, for sharing his laboratory space for the sterile isolation of peripheral blood mononuclear cells and Johanna Acevedo, at the Health Planning Division in the Ministry of Health, Santiago, Chile, for her contributions toward executing this study.

Conflict of interest statement. The authors declare they have no competing interests.

Funding

The research reported in this publication was supported by the National Institute of Environmental Health Sciences of the

National Institutes of Health under Award Nos P42 ES004705 and R01 ES031259. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Ethics statement. Participants provided informed consent, and protocols were approved by the Pontificia Universidad Católica de Chile and the University of California, Berkeley Institutional Review Boards.

Author contributions

A.K.B. analyzed the data and wrote the paper; P.B. analyzed the data, edited, and approved the manuscript; A.E.H. edited and approved the manuscript; F.C.M.S. acquired the data, edited, and approved the manuscript; C.F. acquired the data, edited, and approved the manuscript; C.M.S. conceived the study, designed the study, edited, and approved the manuscript; M.T.S. conceived the study, designed the study, edited, and approved the manuscript; and A.C. designed the study, edited, and approved the manuscript.

References

1. Podgorski J, Berg M. Global threat of arsenic in groundwater. *Science* 2020;**368**:845–50.
2. Naujokas MF, Anderson B, Ahsan H *et al.* The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. *Environ Health Perspect* 2013;**121**:295–302.
3. Bailey KA, Smith AH, Tokar EJ *et al.* Mechanisms underlying latent disease risk associated with early-life arsenic exposure: current research trends and scientific gaps. *Environ Health Perspect* 2016;**124**:170–5.
4. Argos M. Arsenic exposure and epigenetic alterations: recent findings based on the Illumina 450K DNA methylation array. *Curr Environ Health Rep* 2015;**2**:137–44.
5. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet* 2018;**19**:371–84.
6. Steinmaus CM, Ferreccio C, Romo JA *et al.* Drinking water arsenic in Northern Chile: high cancer risks 40 years after exposure cessation. *Cancer Epidemiol Biomarkers Prev* 2013;**22**:623–30.
7. Steinmaus C, Ferreccio C, Acevedo J *et al.* Increased lung and bladder cancer incidence in adults after in utero and early-life arsenic exposure. *Cancer Epidemiol Biomarkers Prev* 2014;**23**:1529–38.
8. Smith AH, Marshall G, Liaw J *et al.* Mortality in young adults following in utero and childhood exposure to arsenic in drinking water. *Environ Health Perspect* 2012;**120**:1527–31.
9. Smith AH, Marshall G, Yuan Y *et al.* Increased mortality from lung cancer and bronchiectasis in young adults after exposure to arsenic in utero and in early childhood. *Environ Health Perspect* 2006;**114**:1293–6.
10. Roh T, Steinmaus C, Marshall G *et al.* Age at exposure to arsenic in water and mortality 30–40 years after exposure cessation. *Am J Epidemiol* 2018;**187**:2297–305.
11. Hochberg Z, Feil R, Constancia M *et al.* Child health, developmental plasticity, and epigenetic programming. *Endocr Rev* 2011;**32**:159–224.
12. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol* 2013;**14**:R115.
13. Hannum G, Guinney J, Zhao L *et al.* Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell* 2013;**49**:359–67.
14. Horvath S, Oshima J, Martin GM *et al.* Epigenetic clock for skin and blood cells applied to Hutchinson Gilford Progeria Syndrome and ex vivo studies. *Aging* 2018;**10**:1758–75.
15. Levine ME, Lu AT, Quach A *et al.* An epigenetic biomarker of aging for lifespan and healthspan. *Aging* 2018;**10**:573–91.
16. Lu AT, Quach A, Wilson JG *et al.* DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging* 2019;**11**:303–27.
17. Ryan J, Wrigglesworth J, Loong J *et al.* A systematic review and meta-analysis of environmental, lifestyle, and health factors associated with DNA methylation age. *J Gerontol* 2019;**75**:481–94.
18. Bozack AK, Boileau P, Wei L *et al.* Exposure to arsenic at different life-stages and DNA methylation meta-analysis in buccal cells and leukocytes. *Environ Health* 2021;**20**:79.
19. Sancha A, O'Ryan R. Managing hazardous pollutants in Chile: arsenic. *Rev Environ Contam Toxicol* 2008;**196**:123–46.
20. Steinmaus C, Ferreccio C, Acevedo J *et al.* High risks of lung disease associated with early-life and moderate lifetime arsenic exposure in Northern Chile. *Toxicol Appl Pharmacol* 2016;**313**:10–5.
21. Fortin J-P, Labbe A, Lemire M *et al.* Functional normalization of 450k methylation array data improves replication in large cancer studies. *Genome Biol* 2014;**15**:503.
22. Houseman EA, Accomando WP, Koestler DC *et al.* DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinform* 2012;**13**:86.
23. Aryee M, Jaffe A, Corrada-Bravo H *et al.* Minfi: a flexible and comprehensive bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics* 2014;**30**:1363–9.
24. Lu AT, Seeboth A, Tsai P-C *et al.* DNA methylation-based estimator of telomere length. *Aging* 2019;**11**:5895–923.
25. Zeileis A, Hothorn T. Diagnostic checking in regression relationships. *R News* 2002;**2**:7–10.
26. Zeileis A, Köll S, Graham N. Various versatile variances: an object-oriented implementation of clustered covariances in R. *J Stat Softw* 2020;**95**:1–36.
27. Zeileis A. Object-oriented computation of sandwich estimators. *J Stat Softw* 2006;**16**:1–16.
28. Zeileis A. Econometric computing with HC and HAC covariance matrix estimators. *J Stat Softw* 2004;**11**:1–17.
29. Champely S. *Pwr: Basic Functions for Power Analysis. R Package Version 1.1-3*. 2015.
30. R Core Team. *R: A Language and Environment for Statistical Computing*. 2015. https://www.r-project.org/ (30 May 2022, date last accessed).
31. Islam K, Haque A, Karim R *et al.* Dose-response relationship between arsenic exposure and the serum enzymes for liver function tests in the individuals exposed to arsenic: a cross sectional study in Bangladesh. *Environ Health* 2011;**10**:64.
32. Peters BA, Liu X, Hall MN *et al.* Arsenic exposure, inflammation, and renal function in Bangladeshi adults: effect modification by plasma glutathione redox potential. *Free Radic Biol Med* 2015;**85**:174–82.
33. Wei S, Zhang H, Tao S. A review of arsenic exposure and lung cancer. *Toxicol Res (Camb)* 2019;**8**:319–27.
34. Oblak L, van der Zaag J, Higgins-Chen AT *et al.* A systematic review of biological, social and environmental factors associated with epigenetic clock acceleration. *Ageing Res Rev* 2021;**69**:101348.
35. Wang C, Koutrakis P, Gao X *et al.* Associations of annual ambient $PM_{2.5}$ components with DNAm PhenoAge acceleration in elderly men: the Normative Aging Study. *Environ Pollut* 2020;**258**:113690.