



Prenatal arsenic metabolite exposure is associated with increased newborn mitochondrial DNA copy number: evidence from a birth cohort study

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Abstract

While mitochondria are susceptible to environmental detriments, little is known about potential associations between arsenic metabolites and mitochondria DNA copy number (mtDNAcn). We attempted to examine whether maternal urinary arsenic metabolite levels in different trimesters were related to neonatal cord blood mtDNAcn. We included 819 mother-newborn pairs embedded in an in-progress birth cohort survey performed from April 2014 to October 2016 in Wuhan, China. We determined maternal urinary arsenic species concentrations in different trimesters. We determined cord blood mtDNAcn using quantitative real-time polymerase chain reaction. In covariate-adjusted models, each one-unit increment of dimethylated arsenic (DMA) and total arsenic (TAs) in the third trimester was related to 8.43% (95% CI 1.13%, 16.26%) and 12.15% (95% CI 4.35%, 20.53%) increases in mtDNAcn, respectively. The dose–response trend with statistical significance was observed across tertiles of DMA and TAs in the third trimester with mtDNAcn (DMA percent changes (% Δ) = 25.60 (95% CI 6.73, 47.82), for the highest vs the lowest tertile ($P = 0.02$); TAs % $\Delta = 40.31$ (95% CI 19.25, 65.10), for the highest vs the lowest tertile ($P = 0.0002$)). These findings may prove the relationships between prenatal arsenic species levels and neonatal mitochondrial dysfunction.

Keywords Arsenic metabolism · Prenatal exposure · Mitochondrial DNA copy number · Cord blood · Repeated measurement

Introduction

Arsenic widely occurs in nature, existing in organic and inorganic forms. Inorganic arsenic (iAs) is generally considered more toxic than organic forms. The World Health Organization has reported that iAs in the groundwater exists at high concentrations ($> 10 \mu\text{g/L}$) in many countries, including Bangladesh, some areas of China (such as Datong and Yuncheng basins in Shanxi, Hetao basin in Inner Mongolia, and Guitun area in Xinjiang), India, Chile, and Mexico (Ravenscroft et al. 2009). Dietary intake has become an essential source of arsenic exposure because of its accumulation in rice, fish, and dairy products (Chain EPoCitF 2009). People from the USA and some European countries have been reported to be exposed to arsenic at levels up to 300 $\mu\text{g/day}$ via food and beverages (European Food Safety 2014; Kurzius-Spencer et al. 2014). iAs could be methylated to organic forms recognized as monomethylated arsenic (MMA) and dimethylated arsenic (DMA). More

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highly methylated arsenicals could be excreted more readily through the urine, decreasing retention and decreased biological exposure and toxicity (Vahter 2002). The urinary arsenic levels could reflect the recently individual iAs absorbed dose, and iAs methylation indices, which could be pretty stable for 810 months, were considered valuable markers for ongoing ingestion iAs (Steinmaus et al. 2005). Nevertheless, the idea has received a challenge considering that methylated trivalent arsenic showed more toxicity than the inorganic form (Stýblo et al. 2002). Arsenic species could cross the placenta (Concha et al. 1998; Hall et al. 2007), resulting in newborns' susceptibility to arsenic exposure (Farzan et al. 2013). Existing epidemiologic research has revealed that early-life exposure to arsenic species was linked to unfavorable birth consequences [(e.g., low birth weight (LBW), preterm birth (PTB) (Gilbert-Diamond et al. 2016; Laine Jessica et al. 2015), poorer neurobehavioral performance and development of newborns (Chen et al. 2023; Liang et al. 2020; Soler-Blasco et al. 2022))] and disease later in life (Chen et al. 2019; Steinmaus et al. 2016). Toxicological evidence demonstrates that exposure to inorganic (Huang et al. 2018; Rodriguez Karina et al. 2016; Rodriguez et al. 2020) and methylated arsenicals (Negro Silva Luis et al. 2017, 2021) could lead to adverse offspring health.

Mitochondria, an essential ingredient of eukaryotic cells, plays a prominent role in many biological processes, like reactive oxygen species (ROS) production, apoptosis, and lipid metabolism (Shadel and Horvath 2015, Wai and Langer 2016). Mitochondria's vulnerability to environmental toxicants might be partially ascribed to its proximity to ROS (Dan Dunn et al. 2015), inadequate repair capacity, deficiency in noncoding introns, and lack of defensive histones (Malik and Czajka 2013). Nevertheless, mitochondria could compensate for genomic insults by modifying their abundance, ultimately altering mitochondrial DNA copy number (mtDNA_{cn}) (Lee and Wei 2000). Generally, mtDNA_{cn}, reflecting the number of copies of the mitochondrial genome per nucleated cell, is a hopeful marker of mitochondrial malfunction (Malik and Czajka 2013). Despite the variation in mtDNA_{cn} among various cell types, mtDNA_{cn} in specific tissues/cells could remain unchangeable due to its rigorous modulation. Accumulating epidemiological studies have associated unexpected mtDNA_{cn} variation from oxidative stress or inflammation with chronic diseases (Hägg et al. 2021). Moreover, studies have demonstrated that mtDNA_{cn} is related to both birth weight and gestational age and is even one of the putative links between poor fetal growth and cardiovascular complications in later life (Gemma et al. 2006; Pejznochová et al. 2008). Recently, mtDNA_{cn} has been proposed as a potential mechanism linking environmental pollutant exposure and reduced birth weight (Barrett Julia 2016). Therefore, identifying the determinants of mtDNA_{cn} may provide more insights into poor fetal growth and

aging-related disease. Recent epidemiological studies have elucidated if arsenic exposure alters mtDNA_{cn} in individuals with different arsenic metabolizing capacity (Ameer et al. 2016; De Loma et al. 2022), which reported that arsenic exposure was associated with increased mtDNA_{cn}, particularly in individuals with less-efficient arsenic metabolism. However, the previous research focused on cross-sectional associations of relatively high levels of arsenic exposure with mtDNA_{cn} among occupational workers and residents of arsenic-contaminated areas, with relatively small samples. Owing to the cross-sectional study design, the critical window of susceptibility to arsenic metabolites is still unknown.

Accordingly, we conducted this study to reveal the relationship between maternal arsenic metabolites concentrations based on repeated measurements and cord blood mtDNA_{cn} in the Chinese population and to identify the critical window of heightened susceptibility to prenatal arsenic metabolite exposure.

Methods

Study population

The analysis population is a subset of the Wuhan Healthy Baby Cohort that has enrolled pregnancies at the first prenatal examination (gestational week less than 16) from 2012 to 2019. Eligibility criteria included fluency in Chinese, singleton pregnancy, and intent to reside in Wuhan throughout gestation. Participants were included in the present analysis if they had available data on (1) maternal urinary arsenic metabolites concentrations and (2) neonatal cord blood mtDNA_{cn}. The present analysis was restricted to 828 mothers recruited between April 2014 and October 2016. We further excluded four women with outliers or missing data on urinary arsenic metabolites concentrations and five women without demographic or clinic information data, leaving 819 pregnant women for this analysis.

Informed written consent was acquired from all participants at recruitment. The Huazhong University of Science Institutional review boards have authorized the research procedures.

Urine acquisition and arsenic metabolite quantification

The periods of maternal urine sample collection at each trimester were at 12–16, 22–26, and 32–36 gestational weeks, respectively. Each participant was required to provide their spot urine sample when they received prenatal care service. The urine samples from the participants were gathered into metal-free polypropylene containers and stored at –20 °C until further analysis. The average gestational

ages (mean \pm standard deviation [SD]) of urine collection for the first, second, and third trimesters were 13.0, 24.0, and 33.3 weeks, respectively. The sample size for each trimester was 815, 812, and 816, respectively. Details of urinary arsenic metabolite quantification are depicted elsewhere (Wang et al. 2021). In short, midstream spot urine samples were gathered during scheduled antenatal visits and stored at $-20\text{ }^{\circ}\text{C}$. Arsenic species were quantified by isotope dilution high-performance liquid chromatography coupled with quadrupole inductively coupled plasma mass spectrometer (HPLC–ICPMS), including arsenobetaine (AsB), arsenite (As³⁺), arsenate (As⁵⁺), MMA, and DMA. Information about quality assurance, including limits of detections (LODs), limits of quantifications (LOQs), and intra-day and inter-day coefficients of variation, is the same as that published by Wang et al. (2021). Specific gravity (SG) was determined to standardize the urinary arsenic species levels.

Relative mtDNAcn measurement

Venous umbilical cord blood was collected in vacutainer tubes containing EDTA immediately after delivery. Before analysis, blood samples were reserved at $-80\text{ }^{\circ}\text{C}$. Relative mtDNAcn measurement was carried out according to a prior described method (Janssen Bram et al. 2012). Information about detailed procedures of quantitative real-time polymerase chain reaction (PCR) is presented in the supporting materials.

Covariates

Maternal demographic data (e.g., maternal age, family annual income, education levels, passive smoking, and folic acid intake) were collected by trained personnel via semi-structured questionnaires. In addition, data on pregnancy (e.g., parity, neonate gender, and pregnancy complications) was retrieved from the medical records. Information about the classification of pre-pregnancy body mass index (BMI) is shown in Supplementary Methods. Secondhand smoke exposure throughout gestation was defined as passive smoke.

Statistical analysis

Continuous variables were expressed as mean \pm SD, whereas categorical variables were expressed as counts (percentage). Arsenic metabolite levels lower than the LOD were replaced with $\text{LOD}/\sqrt{2}$. We used SG to adjust for the dilution to correct urine dilution, which might potentially affect the association of arsenic metabolites with mtDNAcn. The formula of SG normalization is exhibited in the Supplementary Methods.

Urinary arsenic metabolite levels and relative mtDNAcn were log₂-transformed to alleviate the skewed distribution.

Reproducibility of urinary arsenic metabolite concentrations across three pregnancy trimesters as well as any two trimesters was evaluated by the intraclass correlation coefficient (ICC), which was defined as a ratio of the between-person variance to the sum of within- and between-person variances (Rosner 2011). We averaged the SG-adjusted arsenic metabolite levels in different trimesters to generate individual-specific averages throughout gestation—arsenic metabolites. Details about arsenic exposure indices are shown in the Supplementary Methods.

Potential nonlinear associations between arsenic species and cord blood mtDNAcn were examined using 3-knot restricted cubic splines. Except for iAs and mtDNAcn ($P=0.008$), linear relationships were observed between DMA, MMA, TAs, and mtDNAcn ($P>0.05$). Thus, a general linear model was performed to examine the potential connection of individual-specific arsenic metabolite averages (continuous and in tertiles) across three trimesters with cord blood mtDNAcn. Also, generalized estimating equations (GEEs) were performed to assess the relationships of trimester-specific exposures (both continuous and in tertiles) with mtDNAcn (Sánchez Brisa et al. 2011). Tertile cut-points originated from trimester-specific determination, and we defined the lowest tertile as the reference. In addition, we modeled the median value of every tertile to determine the linear trends of these relationships. We converted beta (β) into percent change ($\%\Delta$) of exposures to make the statistical models more interpretable (Barrera-Gómez and Basagaña, 2015). Covariates considered for inclusion were those changed estimates ($\geq 10\%$) in the relationships or those observed to be linked to mtDNAcn in previous studies were based on previous studies. The following variables were included as covariables: maternal age at enrollment, gestational age (weeks), pre-pregnancy BMI (<18.5 , $18.5\text{--}23.9$, $\geq 24\text{ kg/m}^2$), parity history (nulliparous or not), infant sex (male or not), education level (<9 , $9\text{--}12$, ≥ 12 years), passive smoking during pregnancy (yes or no), gestational diabetes mellitus (yes or not), and gestational hypertension disorders (yes or not).

To test the robustness of our results, we completed additional analyses by excluding participants with PTB, LBW, gestational diabetes, or hypertensive disorders. Software SAS (version 9.4, Institute Inc., USA) was used.

Results

Characteristics

Table 1 exhibits the characteristics of the mother-newborn pairs. The mean maternal age at enrollment was 28.94 years. Approximately 68.74% of participating women had a normal pre-pregnancy weight, with an average pre-pregnancy

Table 1 Descriptive characteristics of study participants included in this study ($N=819$)

Population characteristics	N (%) or mean \pm SD
Maternal age (years)	28.94 \pm 3.59
< 25	60 (7.33)
25–34	694 (84.74)
\geq 35	65 (7.94)
Gestational week at labor (week)	39.28 \pm 1.15
Pregnancy weight gain (kg)	16.12 \pm 4.72
< 10	56 (6.84)
10–15	273 (33.33)
16–19	308 (37.61)
> 20	182 (22.22)
Pre-pregnancy BMI (kg/m^2)	20.99 \pm 2.83
Underweight (< 18.5)	144 (17.58)
Normal (18.5–23.9)	563 (68.74)
Overweight (\geq 24)	112 (13.68)
Parity	
Primiparous	660 (80.59)
Multiparous	159 (19.41)
Education (years)	
\leq 9	46 (5.62)
9–12	98 (6.35)
> 12	819 (88.03)
Annual family income (yuan/year)	
< 50,000	88 (10.74)
50,000–99,999	290 (35.41)
\geq 100,000	437 (53.36)
Missing	4 (0.49)
Passive smoke during pregnancy	
No	595 (72.65)
Yes	222 (27.11)
Missing	2 (0.24)
Gestational diabetes mellitus	
No	753 (91.94)
Yes	66 (8.06)
Gestational hypertension disorder	
No	795 (97.07)
Yes	24 (2.93)
Iron supplementation during pregnancy	
No	424 (51.77)
Yes	395 (48.23)
Folic acid supplementation during pregnancy	
No	110 (13.43)
Yes	709 (86.57)
Fetal sex	
Male	439 (53.60)
Female	380 (46.40)
Preterm birth	
Yes	28 (3.42)
No	791 (96.58)
Birth weight (g)	3339.87 \pm 430.87
Cord blood mtDNA ^a	0.92 (1.95)

SD standard deviance, N number, % proportion, BMI body mass index, mtDNA^a mitochondrial DNA copy number

^aGeometric mean (geometric standard deviation)

BMI of 20.99 kg/m^2 . Of the mothers in the analysis sample, 27.11% of women reported exposure to secondhand smoke, 88.03% acquired a college education or above, and 80.59% were primiparous. The proportion of gestational diabetes and hypertension disorders was 8.06% and 2.93%, respectively. For newborns, 53.60% were male, and 3.42% were born preterm.

Distribution of maternal urinary arsenic parameters

Supplementary Table 1 exhibits the concentration profiles of the targets. The target analytes were detected with detection frequencies ranging from 64.2 to 98.7%. The median concentrations of maternal SG-normalized urinary DMA were 8.21, 7.72, and 7.93 $\mu\text{g}/\text{L}$, MMA were 1.10, 1.07, and 1.06 $\mu\text{g}/\text{L}$, iAs were 3.18, 3.66, and 3.82 $\mu\text{g}/\text{L}$, and the TAs were 13.58, 13.59, 13.49 $\mu\text{g}/\text{L}$ in the first, second and third trimester, respectively. Throughout the pregnancy, the ICCs of SG-normalized arsenic metabolites varied from 0.17 to 0.39, indicating poor reproducibility of urinary arsenic species across pregnancy (Supplementary Table 2).

Relationships of maternal arsenic exposure with cord blood mtDNA_{cn}

Figure 1 lists the relationships of average levels of individual arsenic metabolites across three trimesters with the cord blood mtDNA_{cn} alternation (Supplementary Table 3). In crude models, one double increase in DMA and TA levels was related to an 8.56% (95% CI 0.60, 17.16) and 15.94% (95% CI 5.72, 27.14) increase in cord blood mtDNA_{cn}, respectively. In adjusted models, the relationships of maternal DMA and TA levels with cord blood mtDNA_{cn} were still statistically significant (percent difference 9.22%, 95% CI 1.11, 17.98; percent difference 17.21%, 95% CI 6.70, 28.76). Additionally, the highest tertile of DMA concentrations was significantly linked to an approximately 23.97% (95% CI 5.35, 45.89) increment in mtDNA_{cn} compared with the lowest tertile. Moreover, a 35.14% increase of cord blood mtDNA_{cn} was found in the highest tertile of TAs concentration, compared with the lowest tertile (percent difference 35.14%, 95% CI 14.80, 59.10). In the dose–response trend analysis, the positive association between TA level and mtDNA_{cn} remained significant (p trend = 0.0423), yet there was not a remarkable relationship between DMA and mtDNA_{cn} (p trend = 0.0854). Sensitivity analyses did not markedly alter our results (Supplementary Table 4).

Figure 2 (Supplementary Table 5) exhibits the relationships of trimester-specific arsenic metabolite concentrations with cord blood mtDNA_{cn}. In adjusted models, each twofold increment of DMA, iAs, and TAs in the

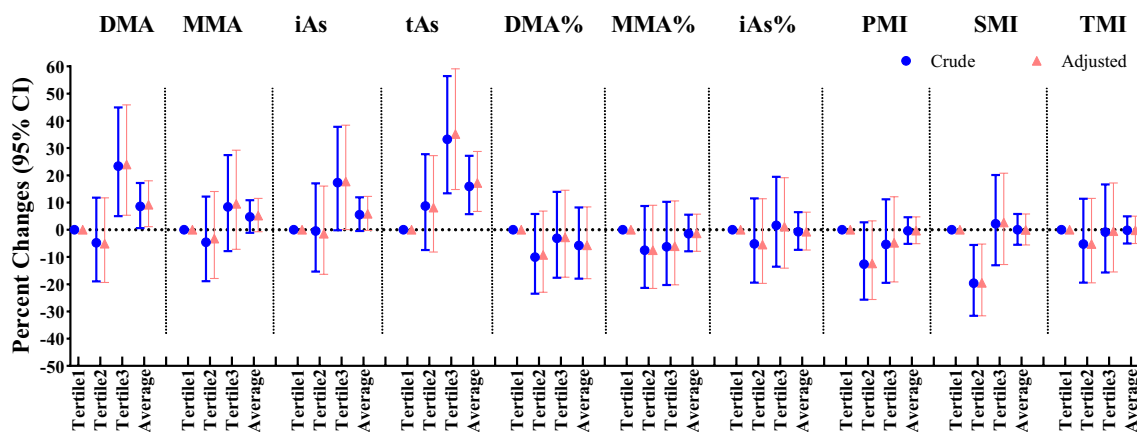


Fig. 1 Associations of average maternal arsenic concentrations with cord blood mtDNAcn (data correspond to Supplementary Table 3). Models were adjusted for maternal age at enrollment, gestational age, pre-pregnancy BMI, parity history, infant sex, education level, passive smoking during pregnancy, hypertensive disorders in pregnancy, and gestational diabetes mellitus. Abbreviations: CI, confidence interval; DMA, dimethylarsinic acid; MMA, monomethylarsonic

acid; iAs, inorganic As, sum of As3+, As5+; TAs, Total As, sum of DMA, MMA, and iAs; %DMA, percentage of dimethylarsinic acid; %MMA, percentage of monomethylarsonic acid; %iAs, percentage of inorganic As; PMI, primary methylation index (MMA/iAs); SMI, secondary methylation index (DMA/MMA); TMI, total methylation index, (MMA + DMA)/iAs. The percentages of each metabolite were calculated: levels of the metabolite/(DMA + MMA + iAs) × 100

third trimester was associated with 8.43% (95% CI 1.13%, 16.26%), 3.94% (95% CI 0.10%, 7.94%), and 12.15% (95% CI 4.35%, 20.53%) increases in mtDNAcn, respectively. Cord blood mtDNAcn increased across increasing tertiles of urinary DMA, iAs, and TAs levels in the third trimester. The observed associations in sensitivity analyses did not change compared with the primary study results (Supplementary Table 6).

Discussion

In this birth cohort study, we demonstrated that prenatal exposure to DMA and TAs during the third trimester, rather than during the first or second trimesters, was positively associated with cord blood leukocyte mtDNAcn. This association persisted even after adjustment for potential confounders.

Arsenic widely exists on the earth, leading to the risk of environmental exposure. It can be converted to MMA and DMA in humans, and urinary arsenic species are acknowledged as a marker of arsenic exposure (Hayakawa et al. 2005). The urinary arsenic speciation levels in this study were lower than those in Mexico (median of DMA, MMA, and TAs were 20.6, 1.4, and 23.3 $\mu\text{g/L}$, respectively) (Laine Jessica et al. 2015) and Pabna (medians of DMA, MMA, and TAs were 50.2, 3.4, and 63.2 $\mu\text{g/L}$) pregnant women (Gao et al. 2019). Additionally, the urinary arsenic speciation levels in this study were higher than those of pregnant women in the INMA Project (geometric means [GM] of DMA, MMA, and iAs were 5.54, 0.28, and 0.27 $\mu\text{g/L}$, respectively) (Soler-Blasco et al. 2021), Oklahoma City

(GM of DMA, MMA, iAs, and TAs were 3.93, <LOD, 1.36, and 4.46 $\mu\text{g/L}$, respectively) (Chen et al. 2021), the PHIME-CROME project (GM of DMA, MMA, and TAs were 2.67, 0.17, and 3.23 $\mu\text{g/L}$, respectively) (Stajniko et al. 2019), and the MADRES study (medians of DMA, MMA, iAs, and TAs were 4.4, 0.4, 0.5, and 5.4 $\mu\text{g/L}$, respectively) (Farzan et al. 2021). Different urinary arsenic speciation levels in other regions might result from variations in research participants, living environments, lifestyle behaviors, dietary intakes, and individual methylation efficiency. Urinary iAs reflect recently personal iAs absorbed dose, and unique iAs methylation patterns remain stable for 8–10 months (Steinmaus et al. 2005). Urinary arsenic concentration could be a surrogate for exogenous multiple exposure sources (Hughes Michael 2006; Kile Molly et al. 2009). Our reported ICCs (0.17–0.39) for repeated urinary arsenic speciation measurements were low, revealing high variability over the three trimesters. The low ICCs might be partly due to the alteration in external exposure level of arsenic speciation or the woman's physiology attributed to the progression of pregnancy (Abduljalil et al. 2012). Given the poor reproducibility in this study, multiple or consecutive measurements along the pregnancy are desired to characterize arsenic speciation exposure during the whole pregnancy accurately.

The mtDNAcn, quantifying mitochondrial genome abundance, is a marker of mitochondrial function and oxidative stress (Castellani et al. 2020a, Malik and Czajka 2013). Mitochondria is the dominant cellular site of oxidative phosphorylation and ROS generation (Meyer et al. 2017). mtDNA is a prime victim of oxidative stress (Yakes and Houten 1997). MtDNAcn in most somatic cells ranges from 100 to 10,000 (Castellani et al. 2020b; Rausser et al. 2021).

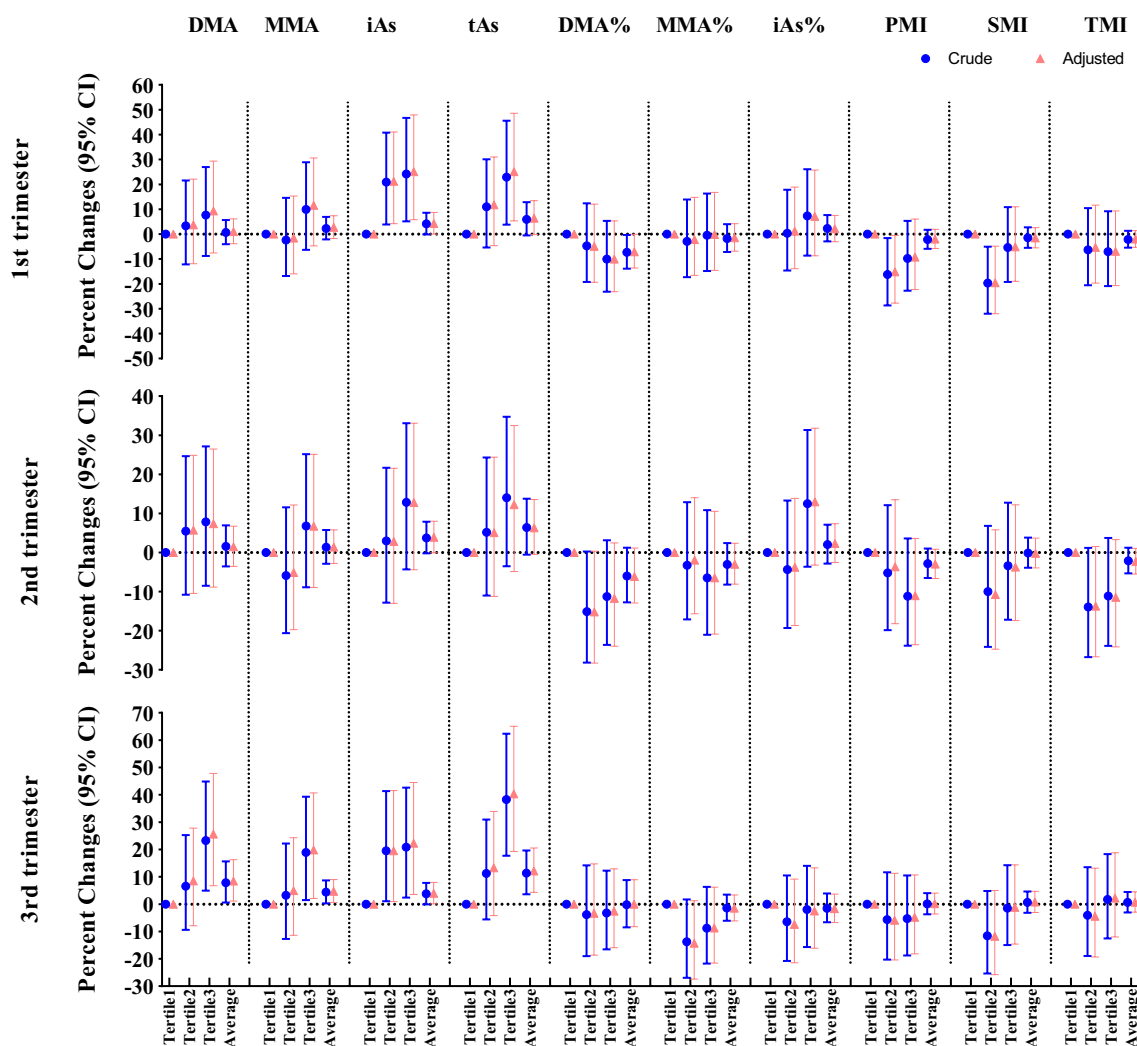


Fig. 2 Trimester-specific associations between prenatal arsenic exposure and cord blood mtDNAcn (data correspond to Supplementary Table 5). Models were adjusted for maternal age at enrollment, gestational age, pre-pregnancy BMI, parity history, infant sex, education level, passive smoking during pregnancy, hypertensive disorders in pregnancy, and gestational diabetes mellitus. Abbreviations: CI, confidence interval; DMA, dimethylarsinic acid; MMA, monomethyl-

larsonic acid; iAs, inorganic As, sum of As^{3+} , As^{5+} ; TAs, Total As, sum of DMA, MMA, and iAs; %DMA, percentage of dimethylarsinic acid; %MMA, percentage of monomethylarsinic acid; %iAs, percentage of inorganic As; PMI, primary methylation index (MMA/iAs); SMI, secondary methylation index (DMA/MMA); TMI, total methylation index, $(MMA + DMA)/iAs$. The percentages of each metabolite were calculated: levels of the metabolite / $(DMA + MMA + iAs) \times 100$

Mitochondria undergo fusion to complement mtDNA lesions via the transfer of nucleoids (Chen et al. 2010). Generally, the quantity of mtDNAcn within a cell is strictly governed to keep the cell in good condition (Attardi and Schatz 1988). Emerging epidemiology evidence has linked variation in mtDNAcn, either elevated or lower levels, to age-related disease (Castellani et al. 2020b; Hägg et al. 2021). Several epidemiological surveys have revealed that toxic exposures during early life were linked to changes in cord blood mtDNAcn. Increased cord blood mtDNAcn was found to be related to prenatal exposure to β -hexachlorocyclohexane (Vriens et al. 2017), aluminum (Liu et al. 2019), manganese (Kupsco et al. 2019), lead, and rare earth elements

(gadolinium, dysprosium, erbium, and praseodymium) (Liu et al. 2020). Other surveys have shown that declined mtDNAcn was related to maternal smoking (Janssen et al. 2017), maternal lifetime stress (Brunst et al. 2017), prenatal exposure to thallium (Wu et al. 2019), arsenic (Song et al. 2020), magnesium (Smith Anna et al. 2021), air pollutants ($PM_{2.5}$, PM_{10} , and NO_2) (Brunst et al. 2018; Clemente Diana et al. 2016; Hu et al. 2020; Janssen Bram et al. 2012), and benzotriazoles (Chen et al. 2020). It is comprehensible that the tendency of mtDNAcn changes varies from various pollutants. Additionally, findings for one exposure were not consistent. Therefore, current epidemiological surveys about the relationships of prenatal exposures with alterations in

child mtDNAcn could not draw firm agreements. This discordance in effects might be partly ascribed to the discrepancies in the characteristics of the study participants, types and levels of exposures, different tissues used to determine mtDNAcn (cord blood vs. placenta tissue), and duration of exposure. Limited research has examined the relationships of maternal arsenic metabolite concentrations with cord blood mtDNAcn.

In this study, we observed positive (urinary DMA and TAs) associations with cord blood mtDNAcn. Prior surveys have shown that people in arsenic-exposed areas have higher mtDNAcn (Ameer et al. 2016; Lou et al. 2022; Sanyal et al. 2018). To our knowledge, only one study in Belgium has shown that cord blood TAs were linked to an elevated placental mtDNAcn (Vriens et al. 2017). Increased mtDNAcn manifests enhanced mitochondrial biogenesis resulting in cell aberrant proliferation (Lee et al. 2011, Malik and Czajka 2013, Wei and Lee 2002). Previous experimental surveys have displayed that low-level arsenic exposure enhanced mitochondrial biogenesis (Lee et al. 2011; Lou et al. 2022). Lee et al. found that primary keratinocytes treated with arsenic at concentrations lower than 1.0 $\mu\text{mol/L}$ exhibited increased cell proliferation and mitochondrial biogenesis (Lee et al. 2011). Luo et al. found mtDNAcn in human hepatocyte L-02 cells treated with a low dose of iAs^{3+} (0.2 $\mu\text{mol/L}$) increased by more than 50% (Lou et al. 2022). Some surveys have reported that mtDNAcn increases with ROS to compensate for mtDNA lesions (Lee et al. 2000a; Liu et al. 2006; Wei et al. 2001). As a noted ROS stimulus, arsenic induces several types of ROS production, like peroxyl radicals, superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide (Banerjee et al. 2011; Lynn et al. 2000). Little research has documented arsenite's capability to promote mitochondrial ROS generation (Liu et al. 2005; Partridge et al. 2007). When ROS damaged mtDNA, reinforced mitochondrial biogenesis could increase mtDNAcn to make amends for mitochondrial malfunction (Giordano et al. 2014; Lee et al. 2000b). Nevertheless, excessive ROS might bring about declined mtDNA synthesis (Hou et al. 2010). Maintaining mtDNAcn in response to ROS induced by different pollutants remains unclear. Further surveys based on prospective cohorts and ingeniously designed toxicological surveys are needed to elucidate the toxic mechanism for arsenic species. Our findings also showed a possibly enhanced susceptibility to arsenic species exposure in the third trimester. Probable explanations might be variations in arsenic metabolite concentrations across pregnancy or chronic arsenic toxicity in mitochondria (Partridge et al. 2007). More studies are warranted to determine possible windows of prenatal exposure to arsenic species.

So far, this is the first report on connections between maternal arsenic species and cord blood mtDNAcn, using repeated measurements. Notwithstanding, some

disadvantages could not be ignored. First, it was not possible to eliminate the residual confounding by other unmeasured factors, like the lack of cell-type data for cord blood mtDNAcn. Blood samples used to quantify mtDNAcn consist of different cell types, and mtDNAcn in diverse immune cells vary (Hurtado-Roca et al. 2016). Equally, mtDNAcn is estimated according to genomic DNA originating from the buffy coat, which is confounded by both cell type distributions and contaminating platelets (Rausser et al. 2021). The lack of data on platelet count in the cord blood samples might result in the overestimation of leukocyte mtDNAcn quantification in consideration of high levels of mtDNA, not nuclear DNA, in platelets (Hurtado-Roca et al. 2016; Urata et al. 2008). Second, oxidative stress biomarkers in cord blood samples were not determined, which were associated with mitochondrial function (Liu et al. 2003). Finally, all participants in this survey were Chinese, which limited external generalizability to other populations. Further surveys of different people are suggested to confirm our findings.

Conclusion

In this prospective pre-birth cohort from Wuhan, China, DMA and TA exposure in the third trimester was linked to elevated cord blood mtDNAcn. Our findings provided epidemiological data on mtDNA as a target of metals and emphasized a possible complicated part of arsenic specie in pathways of disease. Additional surveys are demanded to shed light on the molecular pathways involved in cord blood mtDNA damage induced by maternal methylated arsenic exposure.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11356-024-32933-7>.

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Author contribution Feng Qiu: conceptualization, data curation, methodology, software, formal analysis, writing—original draft; Hongling Zhang: data curation, formal analysis; xin Wang: investigation, methodology; Zhenxian Jia: data curation, validation; Yujie He: investigation, methodology, validation; Yi Wu: investigation, methodology; Zhangpeng Li: data curation, investigation; Tongzhang Zheng: data curation, investigation. Wei Xia: project administration, data curation, supervision. Shunqing Xu: conceptualization, project administration, resources; Yuanyuan Li: conceptualization, funding acquisition, project administration, writing—review and editing.

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Data availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate This study was evaluated and approved by the Huazhong University of Science Institutional review boards.

Consent for publication All authors revised the manuscript critically for intellectual content, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Competing interests The authors declare no competing interests.

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