



Effects of exposure to environmental pollutants on mitochondrial DNA copy number: a meta-analysis

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Abstract

Exposure to environmental pollutants has been associated with alteration on relative levels of mitochondrial DNA copy number (mtDNA_{cn}). However, the results obtained from epidemiological studies are inconsistent. This meta-analysis aimed to evaluate whether environmental pollutant exposure can modify the relative levels of mtDNA_{cn} in humans. We performed a literature search using PubMed, Scopus, and Web of Science databases. We selected and reviewed original articles performed in humans that analyzed the relationship between environmental pollutant exposure and the relative levels of mtDNA_{cn}; the selection of the included studies was based on inclusion and exclusion criteria. Only twenty-two studies fulfilled our inclusion criteria. A total of 6011 study participants were included in this systematic review and meta-analysis. We grouped the included studies into four main categories according to the type of environmental pollutant: (1) heavy metals, (2) polycyclic aromatic hydrocarbons (PAHs), (3) particulate matter (PM), and (4) cigarette smoking. Inconclusive results were observed in all categories; the pooled analysis shows a marginal increase of relative levels of mtDNA_{cn} in response to environmental pollutant exposure. The trial sequential analysis and rate confidence in body evidence showed the need to perform new studies. Therefore, a large-scale cohort and mechanistic studies in this area are required to probe the possible use of relative levels of mtDNA_{cn} as biomarkers linked to environmental pollution exposure.

Keywords Mitochondrial DNA copy number · Environmental pollutants · Biomarker · Meta-analysis · A systematic review

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Introduction

Mitochondria regulate several vital functions for maintaining cellular homeostasis and cell survival; some key processes are ATP production based on oxidative phosphorylation, cell signaling for controlling cell death by apoptosis, and others. Mitochondria possess their DNA; the mitochondrial DNA (mtDNA) is a circular double-stranded DNA molecule. Each mitochondrion contains several copies of mtDNA located in the mitochondrial matrix; mtDNA encodes 13 protein subunits implicated in oxidative phosphorylation, two rRNAs, and 22 tRNAs (Barshad et al. 2018). It is widely accepted that the mtDNA copy number (mtDNA_{cn}) specifically varies according to cell or tissue types (Herbers et al. 2019).

Toxicity starts with the interaction between the toxicant and its target molecule. It occurs when the ultimate toxicant reacts to an effective concentration at the target site. The target molecule has three key characteristics: (1) the capacity to react with the ultimate toxicant (2) that results in a disturbing function (3) which is mechanistically related to

toxicity (Lehman-McKeeman and Armstrong 2022). In this direction, mitochondria could be considered a target molecule linked with or responsible for toxicity; it possesses several characteristics that increase the susceptibility to toxicity associated with environmental pollutant exposure. For example, mitochondria have double membrane rich in phospholipids that could attract lipophilic toxicants; mitochondria's monooxygenases could participate in the bioactivation of environmental pollutants; metallic environmental pollutants could input cross calcium channels present in the mitochondria, and the gradient of protons in the intermembrane space, and the pH of the mitochondrial matrix could promote the accumulation of cationic metals and amphiphilic environmental pollutants (Meyer et al. 2013, 2017, 2018).

Consequently, several biomarkers have been suggested to evaluate human health linked to mitochondrial health. Early studies showed that deletions and mutations on mtDNA resulted in several complex diseases denominated as mitochondrial diseases. Interestingly, diagnosed individuals with the same mitochondrial disease show different levels of mtDNAcn; both increase and decrease in mtDNAcn were related with severe consequences in the clinical manifestations of mitochondrial disease (Bai et al. 2004). This observation indicated the possible use of mtDNAcn levels as a potential biomarker related to human health. In this context, a variation on mtDNAcn has been primarily studied as a possible predictor for the development of human diseases such as cancer, cardiovascular, kidney, liver, and neurodegenerative diseases (Castellani et al. 2020).

Several environmental pollutants can induce mitochondrial toxicity resulting in oxidative stress, altered mitochondrial ATP production, and disrupted mitochondrial-mediated cell signaling (Meyer et al. 2018). Moreover, early studies reported that environmental pollutants such as PAHs showed a high affinity to mtDNA compared to nuclear DNA (Allen and Coombs 1980). For all these reasons, the determination of changes on mtDNAcn has been monitored in response to environmental pollutant exposure. *In vitro*, *in vivo*, and epidemiological studies have reported a direct relationship between the alteration in mtDNAcn, and the exposure to environmental pollutants; nevertheless, the epidemiological studies based on environmental or occupational exposure to environmental pollutants showed contradictory results (Roubicek and Souza-Pinto 2017).

On the other hand, the implementation of systematic reviews focused on environmental issues allows assessment of the state of science concerning a particular topic. Moreover, the derived results are reproducible, transparent, formal, and accurate. Therefore, systematic reviews in environmental epidemiology are employed to make evidence-based decisions in the health environment (Rooney et al. 2014). Moreover, recently, a specific tool has been developed for performing a systematic review and meta-analyses

in environmental epidemiology; this tool is used to evaluate the risks of bias, assess the quality of individual studies, and rate confidence in the body of evidence (NTP/OHAT 2019). Hence, this systematic review and meta-analysis aimed to determine the pooled effect of exposure to environmental pollutants on changes in mtDNAcn.

Materials and methods

To carry out this work, we followed the recommendations from the Handbook for Conducting a Literature-based Health Assessment Using OHAT Approach for Systematic Review and Evidence (NTP/OHAT 2019) and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al. 2021). The PRISMA checklist is presented in supplementary data 4. A protocol was developed and registered (registration number CRD42020211134) in the international prospective register of systematic review (PROSPERO) database. A systematic search of the literature up to Oct 2020 was performed. The search was performed using PubMed, Scopus, and Web of Science databases, using the following keywords: “Environmental Pollutants” OR “Environmental Pollution” and “DNA, Mitochondrial.” We used the Medical Subject Headings (MeSH) and the most frequent synonyms. In addition, the search also included the review of the cited bibliographies to identify omitted articles.

Evidence searches

We followed the PECO (population, exposure, comparators, and outcomes) strategy for the reach question construction and evidence search (da Costa Santos et al. 2007). The critical elements of the PECO strategy are presented in Table 1.

Selection criteria

The selection criteria were as follows: (1) original articles (2) performed in humans (case-control, cross-sectional, or cohort studies) that (3) evaluated the association between mtDNAcn and the exposure to environmental pollutants. (4) The result of interest was the mtDNAcn expressed as the mean and standard error (or the data for their estimation).

The exclusion criteria were as follows: (1) review articles, letters, comments, correspondence, and conference reports; or (2) studies that did not provide enough data. In addition, we selected the most recent or most comprehensive manuscript if duplicate reports from a research group existed. Finally, if the authors only reported a graphic without descriptive data, we analyzed the reported graphic using the “WebPlotDigitizer—Extract data from plots, images, and maps” (version 4.0 PLOTCON 2017-Oakland, USA)

Table 1 Description of the PECO strategy

Population	Exposure	Comparative	Outcome
Individuals without regard to their anthropometric characteristics, age, country of residence, or sociodemographic characteristics	To quantify and confirm exposure to environmental pollutants, we considered all biological matrices. Furthermore, the verification of exposure may have been achieved by self-report (occupational exposure)—alternatively, the direct or indirect measure of the environmental pollutant or its metabolites. In addition, the assessment of effect biomarkers or exposure biomarkers was considered	The comparison group had to be the population non-occupational exposed or exposed to lower levels of environmental pollutants	Primary outcome: changes in the relative levels of mtDNAcn

for obtaining the descriptive data. Unselected articles that reported the effect of human exposure to environmental pollutants on mtDNAcn are presented in supplementary data 5.

Data extraction

For the included studies, we extracted the following data: family name of the first author, publication year, study design, geography localization, sample size, sex of participant, age, environmental pollutant, the employed method for exposure assessment, and biological matrix used for exposure assessment, confounding variables, and statistical methods. In addition, for all included studies, the information regarding the measure of mtDNAcn such as the method used for relative quantification, the mitochondrial and nuclear genes used in the assay, and the biological matrix used for the obtained DNA was extracted from each study. Two researchers independently selected the studies (CAR, MARV). Differences in study selection between researchers were infrequent and resolved by consensus.

Assessment of risk of bias

Two independent reviewers performed the quality assessment for each included study. The OHAT's risk of bias tool for human and animal studies was used as a standardized method to quantify the risk of bias (NTP/OHAT 2019). The tool was designed for global selection-, confounding-, and detection-bias assessments. In brief, risk of bias questions addressed similarity between compared groups, confounder's consideration (methodological measure and statistical treatment), pollutant exposure characterization (use of well-established methods; limits of detection and quantification reported and not near values), and outcome assessment (use of well-established methods, coefficient of variation reported, use of standards or internal controls, the blinding during the analysis). We rank studies by reported information in "Definitively low risk of bias," "Probably low risk of bias," "Probably high risk of bias," or "Definitively high risk of bias."

Statistical analysis

We calculated the standardized mean differences (SMDs) with 95% confidence intervals (95% CIs) of mtDNAcn in each study using a random-effects model. The random-effects analysis assumes that the true effect could vary between the studies and that the studies analyzed in the meta-analysis are a random sample of the effects that could be observed (Borenstein 2009). We considered the high versus low level of exposure for studies that reported categorical exposure. The I^2 test was used to measure the proportion of variation between studies concerning total

variation “heterogeneity.” For the I^2 test, a value of 0% indicates no observed heterogeneity. The values between 25 and 50% indicate a low heterogeneity, and the values between 50 and 75% indicate a moderate heterogeneity, while the values $\geq 75\%$ indicate a high heterogeneity (Higgins and Thompson 2002). In addition, the publication bias was evaluated by visual inspection of Egger’s publication bias plot, and the effect of small studies was tested using Egger’s test (Pustejovsky and Rodgers 2019). Statistical analyses were performed using the statistical package STATA version 16 (College Station, TX).

Trial sequential analysis

The meta-analyses are considered the best available evidence. However, the results derived from meta-analytic techniques could be influenced by statistical errors such as type 1 or 2. For this reason, it is essential to employ methods for ensuring that obtained results have a negligible risk of reporting false-positive or false-negative results. Trial sequential analysis (TSA) is used to decrease the false-positive or false-negative risk. TSA estimates the necessary number of events and participants to calculate a reliable statistical inference. In addition, the strength of evidence is measured using the accrued number of participants, observed difference of outcome measured in the included trials, and the impact of assortment. If the cumulative Z-curve crosses the TSA limit, it is inferred that the meta-analysis has enough evidence.

On the other hand, if the Z-curve does not cross the TSA limit, it is inferred that there is not enough evidence, and more trials are necessary (Shah and Smith 2020; Wetterslev et al. 2017). We examined the reliability and confidence of the available reports using TSA. We performed TSA analysis considering an overall 5% risk of a type I error and 20% of a type II error (power of 80%). The sequential trial analysis was performed using TSA version 0.9.5.10 β (“TSA”).

Rate the confidence in the body evidence

The OHAT method based on the Grading of Recommendations Assessment, Development and Evaluation “GRADE” was employed for rating the confidence in the body evidence (NTP/OHAT 2019). The GRADE tool starts evaluating the initial confidence rate considering the study design; human controlled trials are considered with a high initial confidence rating, and other studies such as case–control/cohorts or cross-sectional studies are evaluated with low-to-moderate initial confidence rate. After the first assessment, key factors can change the rate of confidence. Factors downgrading the confidence are the following: (1) risk of bias (the proportion of the included studies classified as T1 to T3 is considered); (2) imprecision (for continuous outcomes downrating

is considered if the included studies reported sample sizes lower than 400); (3) publication bias (identified using funnel plots and Egger’s regression); (4) indirectness and applicability (epidemiological studies are not downgraded for directness regardless of the exposure level); (5) unexplained inconsistency (downgrading if significant and considerable heterogeneity is presented in meta-analysis).

Results

Literature search results

The electronic database searches yielded 465 records, and 69 records were identified from reviewing the referent list of the collected record. One hundred seven records were reviewed at the full-text level because of title and abstract screening. However, only twenty-two records fulfilled our inclusion criteria and were included in the meta-analysis (Fig. 1). Furthermore, in supplementary data 5, we summarized the unselected articles that evaluated the effect of human exposure to environmental pollutants on mtDNAcn.

Study characteristics

Table 2 summarizes the main characteristics of the included studies. Most included studies ($\approx 82\%$) were performed under a cross-sectional design, and two studies reported results from prospective cohorts. In addition, a matched case–control or longitudinal design was performed by Lin-qing et al. (2016) and Hou et al. (2010), respectively. Fifty-five percent of the included studies in the meta-analysis captured both men and women; 32% of the studies only had men. Only one study did not report the sex of the included participants, and the remaining studies were performed on women.

Considering all age groups reported by the included studies, 95% of studies had adults, and only one study was performed on children. Seventy-three percent of the included studies measured the mtDNAcn using the ND1 as referent mitochondrial gene and β -globin as referent nuclear gene. The whole blood or isolated leukocytes was the most frequent (73.9%) biological matrix used by DNA isolation. Only 27% of the included studies did not conduct an adjustment for confounding factors. Other characteristics from included studies are shown in supplementary data 1.

Other characteristics such as recruitment strategy, inclusion, and exclusion criteria for the included participants, the method employed for exposure assessment, the instrument used (with limits of quantification or detection), and the standards employed are presented in supplementary data 1. The meta-analysis includes 6011 individuals exposed to environmental pollutants.

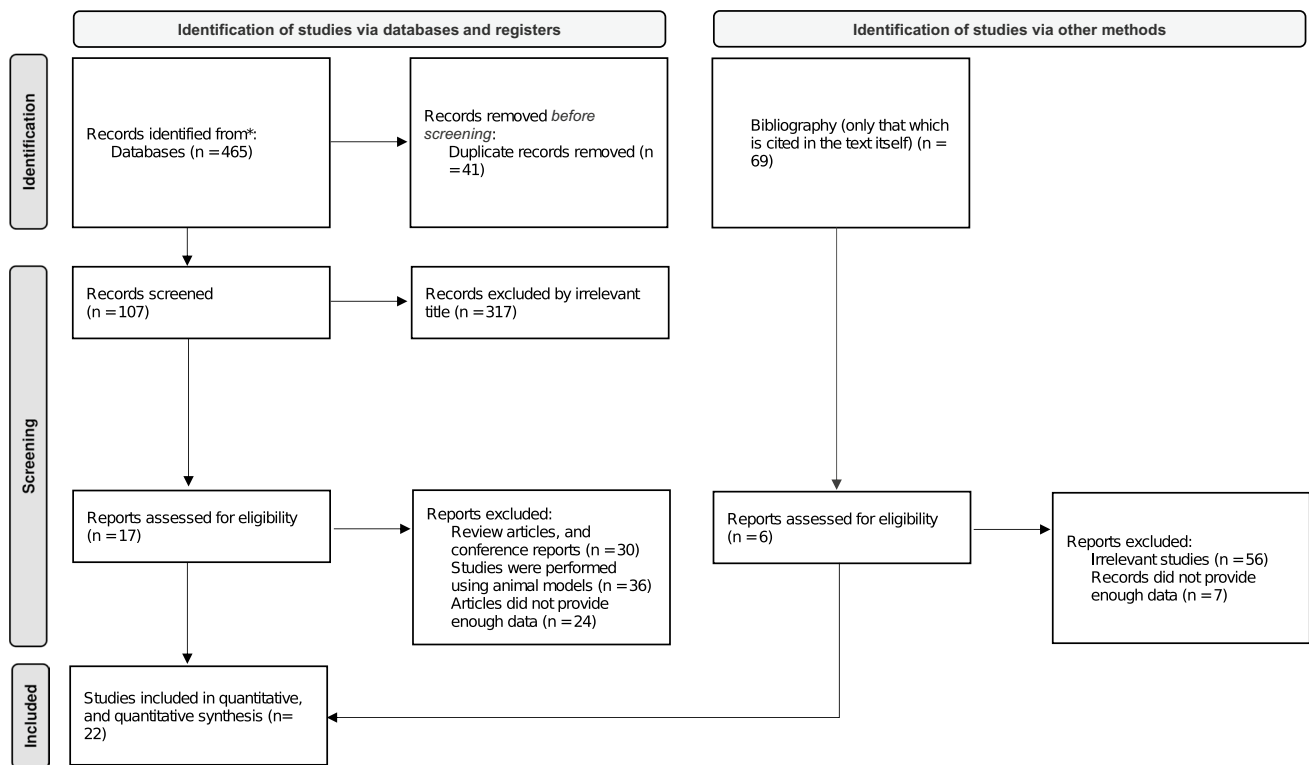


Fig. 1 Search strategy and study selection process

We grouped the included studies according to the type of environmental pollutant. We considered a conventional classification based on similar toxic mechanisms and physicochemical characteristics. We have grouped the included articles into four main groups. The heavy metal group includes metallic chemical elements characterized by their relatively high density and their toxic effect at low concentrations (Balali-Mood et al. 2021). Benzene and polycyclic aromatic hydrocarbons were grouped. Polycyclic aromatic hydrocarbons (PAHs, sometimes referred to as polynuclear aromatic hydrocarbons (PNAs)) are a large group of diverse organic compounds, which contain two or more fused aromatic rings. At the same time, benzene has only one aromatic ring (Fan and Lin 2011). Another group is comprised of articles that evaluate human exposure to particulate matter. Particle pollution is a general term for a mixture of solid and liquid droplets suspended in the air (Ukaogo et al. 2020). And finally, the fourth group is comprised of articles that evaluated the active exposure to cigarette smoke.

Effect of heavy metal exposure on the mtDNAcn

Alegría-Torres et al. (2020) characterized the concentrations of arsenic (As) and lead (Pb) in children from schools close to an oil refinery and thermoelectric plant.

However, the author only reported the changes of mtDNAcn according to Pb blood concentration. Ameer et al. (2016) reported the changes of mtDNAcn according to inorganic As and its metabolites such as methylarsonic acid (MMA) and dimethylarsinic acid (DMA) in urine; the exposure to As occurred via the drinking water consumed by residents. Li et al. (2020a, b) included participants exposed to electronic waste; the reported results indicated that the concentrations of As, nickel (Ni), silver (Ag), lanthanum (La), and cerium (Ce) were higher in individuals exposed to electronic waste. Linqing et al. (2016) conducted a matched case–control study; they measured chromium (Cr), zinc (Zn), copper (Cu), cadmium (Cd), and selenium (Se) concentrations from blood in the exposed and control groups. Nevertheless, only the Cr concentration was higher in the exposed group.

The mtDNAcn showed an increase in highly exposed individuals to As, Cr, or electronic waste. An inverse effect was observed in the highly exposed individuals to Pb. Although the pooled analysis showed a marginal and insignificant increase in the mtDNAcn, the pooled standardized mean difference was 0.72-fold higher in highly exposed individuals (95% CIs: -0.30 to 1.75 ; $Z = 1.39$; $p = 0.17$) (Fig. 2a). High and significant heterogeneity was observed ($I^2 = 96.7\%$, $p < 0.0001$).

Table 2 Characteristics of the included studies in the systematic review and meta-analysis

Reference	Study design		Country	Sample size	Sex	Age	Exposure assessment		Outcome assessment		Confounders
	Method	Pollutant					Matrix	MG/NG	Matrix		
Alegria-Torres et al. (2020)	CS	Mexico	88	FM	9 ± 1.63	As/Pb	ICP-MS	Urine/Blood	MT-ND1/β-GLOBIN	IL	Sex; age; TLC
Ameer et al. (2016)	CS	Argentina	433	FM	37 ± 9.3	As	HPLC-ASS/HPLC-HG-ICPMS	Urine	MT-ND1/β-GLOBIN	WB	Sex; age
Bouhours-Nouet et al. (2005)	CS	France	29	F	NA	Cigarette	Self-report	NA	NA/β-Globin	Placenta	
Carugno et al. (2012)	CS	Italy	519	FM	39 ± 11.8	Airborne benzene	GC/MS	The near “breathing zone” of study participants	MT-ND1/β-GLOBIN	WB	Sex; age; SM; NCD
Du et al. (2020)	CS	China	697	FM	39.7 ± 9.7	PAHs	HPLC-MS/MS	Urine	MT-ND1/β-GLOBIN	WB	Sex; age, education; SS, AS, TDS, ES; NSS
Duan et al. (2020)	CS	China	782	FM	39.6 ± 7.1	PAHs	Gravimetry	Urine	MT-ND1/β-GLOBIN	WB	Sex; age; BMI; SM; DK
Hou et al. (2013)	CS	China	120	FM	32 ± 7.6	PM _{2.5} and PM ₁₀	Gravimetry	The near “breathing zone” of study participants	MT-ND1/β-GLOBIN	BC	Sex; age; BMI; SM; PYCS
Hou et al. (2010)	LNG	Italy	63	M	43.5 ± 6	PM ₁ and PM ₁₀	Scattering dust analyzer	Airborne from work areas	MT-ND1/β-GLOBIN	BC	Age; BMI; SS; YS; PN
Lee et al. (1998)	CS	Taiwan	49	ND	48 ± 15.4	Cigarette	Self-report	NA	MT-ND1/β-GLOBIN	LT	
Li et al. (2020a, b)	CS	China	150	FM	42.6 ± 5.6	MFEW	ICP-MS	Plasma, blood, or hair	NA/β-Globin	WB	
Li et al. (2020a, b)	CS	China	232	M	43.2 ± 8	Benzene	GC	The respiratory zone of each worker	MT-ND1/β-GLOBIN	WB	Age; BMI, SM; AS
Ling et al. (2017)	CS	China	666	M	21 ± 0.9	PAHs	HPLC-MS/MS	Urine	16S rRNA/GAPDH	Semen	Age; BMI; SS; CA; BF; DA
Linqing et al. (2016)	MCC	China	58	FM	41.8 ± 8.6	Cr, Cd, Cu, Zn, Mg, and Ca	ICP-MS	Blood	MT-ND1/β-GLOBIN	WB	Sex; age; SM; DH
Masayeva et al. (2006)	CS	USA	604	FM	62.8 ± 12.4	Cigarette	Self-report	NA	Coxi/β-actin	UATM	
Pavanello et al. (2013)	CS	Poland	90	FM	37.4 ± 8.7	PAHs	HPLC-MS/MS	Urine	MT-ND1/β-GLOBIN	WB	Age
Shen et al. (2008)	CS	China	80	FM	32.5 ± 9.3	PAHs	ND	Urine	MT-ND1/β-GLOBIN	leu	RI
Tan et al. (2008)	CS	USA	72	FM	45.8 ± 13.8	Cigarette	Self-report	NA	Col/β-actin	BCs; Ly	

Table 2 (continued)

Reference	Study design	Country	Sample size	Sex	Age	Exposure assessment		Outcome assessment		Confounders	
						Pollutant	Method	Matrix	MG/NG		Matrix
Wang et al. (2020)	CO	China	120	M	40.6 ± 2.8	PM _{2.5} , PM ₁₀ , SO ₂ , NO ₂ , and CO	Environmental Monitoring Centre	NA	MT-ND1/β-GLOBIN	WB	
Wong et al. (2017)	CS	China	148	F	55 ± 14.4	PM _{2.5} and Benzene	GC/MS/Gravimetry	The breathing zone of each participant	MT-ND1/β-GLOBIN	WB	Age; BMI; FT
Xu et al. (2017)	CS	Sweden	228	M	42 ± 7	Welding fumes	Gravimetry	Respirable dust from the workers breathing zone	MT-ND1/β-GLOBIN	WB	Age; BMI; PSY; STS; CR
Xu et al. (2018)	CS	Sweden	267	M	43.9 ± 7.5	PAHs	LC-MS/MS	Urine	MT-ND1/β-GLOBIN	WB	Age; BMI; PSY; STS; CR
Zhang et al. (2020)	CO	China	516	M	21 ± 0.6	PM ₁₀ , PM _{2.5} , SO ₂ , NO ₂ , O ₃ , CO	Environmental Monitoring Centre	NA	16S rRNA/GAPDH	Semen	Age; BMI; SM; DS; DA; coffee; localization; AAT

Study design: CS, cross-sectional; CO, cohort; MCC, matched case-control; LNG, longitudinal

Sex: F, female; M, male

Exposure assessment

Pollutant: As, arsenic; Pb, lead; PAHs, polycyclic aromatic hydrocarbons; PM_{2.5}, particulate matter 2.5; PM₁₀, particulate matter 10; PM₁, particulate matter 1; Cr, chrome; Cd, cadmium; Cu, copper; Zn, zinc; Mg, magnesium; Ca, calcium; MFEW, metals from electronic waste

Method: ICP-MS, inductively coupled plasma mass spectrometry; GC/MS, coupling gas chromatography to mass spectrometry; HPLC-MS/MS, high-performance liquid chromatography with electrospray triple-quadrupole tandem mass spectrometry; LC-MS/MS, liquid chromatography coupled to tandem mass spectrometry; HPLC-ASS, high-performance liquid chromatography-atomic absorption spectrometry; HPLC-HG-ICPMS, high-performance liquid chromatography-hybrid generation and inductively coupled plasma mass spectrometry; GC, gas chromatography

Outcome assessment: MG, mitochondrial genes; NG, nuclear gen; MT-ND1, mitochondrially encoded NADH: ubiquinone oxidoreductase core subunit 1; β-GLOBIN, human beta-globin; Col, cytochrome c oxidase I; IL, isolated leukocytes; WB, whole blood; BC, buffy coat; LT, lung tissues; UATM, upper aerodigestive tract mucosa; BCs, buccal cells; Leu, leukocytes; L, lymphocytes

Confounders: BMI, body mass index; TLC, total leukocyte count; SM, smoking; DK, drinking; NCD, number of cigarettes per day; PYCS, pack-year of cigarette smoking; SS, smoking status; YS, year of smoking; PN, percent neutrophils; CA, consumption of alcohol; BF, baked foods; DA, duration of abstinence; DH, drinking history; RI, recent infection; FT, fuel type; PSY, previous smoking years; CR, current residence; STS, smokeless tobacco “snus” status; DS, drinking status; AS, average ambient temperature; AAT, tea drinking status; TDS, tea drinking status; ES, exercise status; NSS, night shift status

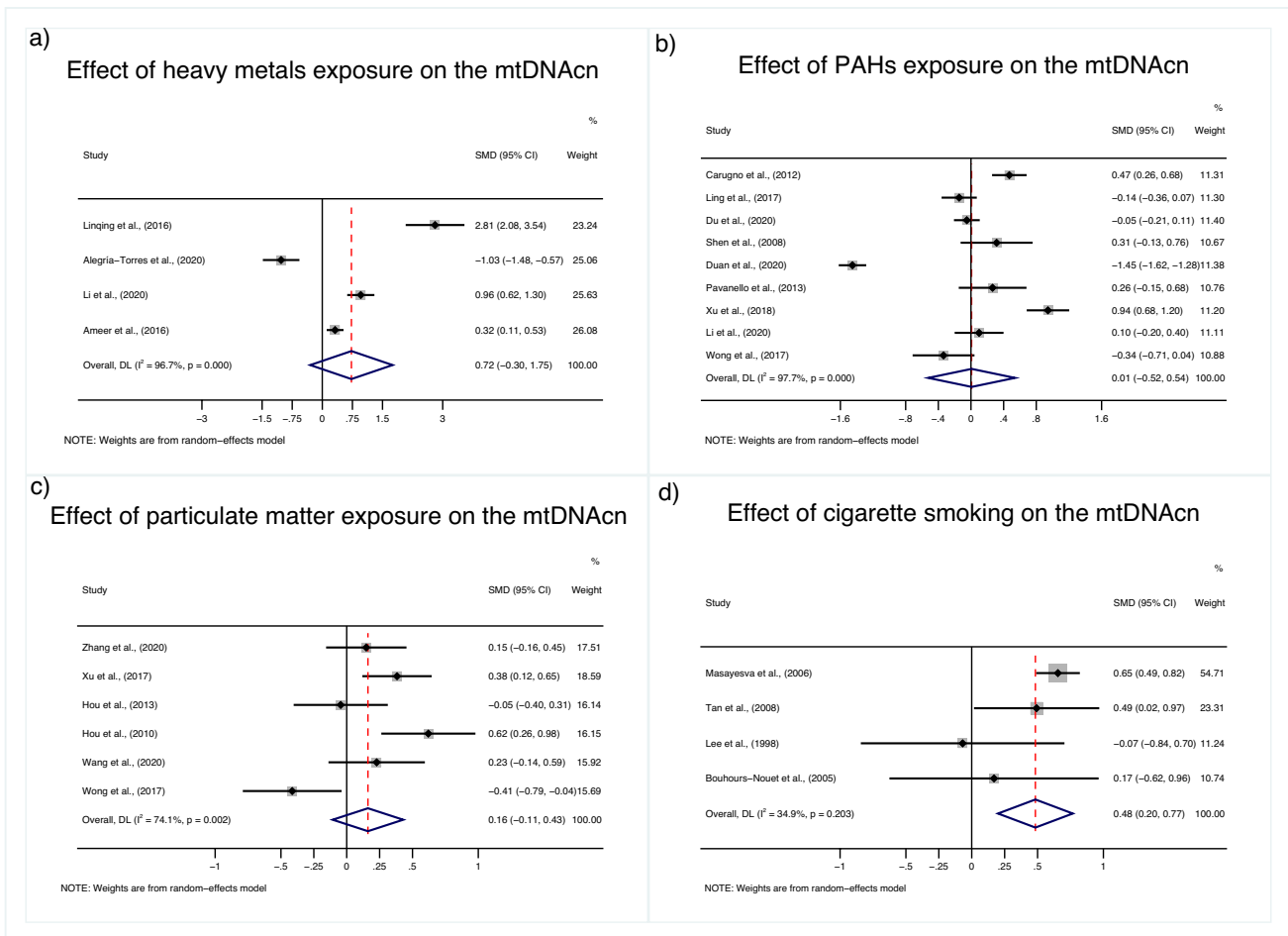


Fig. 2 Forest plot estimates for analyzing the changes in mtDNAcn induced by the exposure to **a** heavy metals, **b** benzene and polycyclic aromatic hydrocarbons, **c** particulate matter, and **d** cigarette smoking. The standardized mean differences (SMD) with the 95% CIs are presented. The size of the gray box is proportional to the weight assigned

Effect of benzene and polycyclic aromatic hydrocarbon exposure on the mtDNAcn

The changes in the mtDNAcn in response to PAH exposure were analyzed by Du et al. (2020), Duan et al. (2020), Ling et al. (2017), Pavanello et al. (2013), Shen et al. (2008), and Xu et al. (2018); and the effect to exposure to benzene on mtDNAcn was analyzed by Carugno et al. (2012), Li et al. (2020a, b), and Wong et al. (2017). However, the studies were performed on heterogeneous populations. For example, Du et al. (2020), Duan et al. (2020), and Pavanello et al. (2013) recruited coke-oven workers occupationally exposed to PAHs. The effect of occupational exposure to benzene on mtDNAcn was reported by Carugno et al. (2012), while other studies included individuals exposed to a complex mixture of fumes delivery

to each study, and horizontal lines represent the 95% CIs. The blue diamond indicates the overall effect size, the width of the blue diamond represents the 95% CIs, and the vertical dashed line represents the pooled error standard

from the combustion of solid fuels (Wong et al. 2017) or emit from hot asphalt (Xu et al. 2018). Only one study reported results from a cohort study (Ling et al. (2017)). The exposure assessment was performed measuring the PAH metabolite concentrations from the urine of the included individuals (Du et al. 2020; Ling et al. 2017; Pavanello et al. 2013; Xu et al. 2018) or quantifying the airborne PAHs from the breathing zone of everyone (Carugno et al. 2012; Duan et al. 2020; Li et al. 2020a, b; Shen et al. 2008; Wong et al. 2017).

A pooled analysis showed that the mtDNAcn was 0.008-fold higher in the highly exposed individuals than in the lowly exposed group (95% CIs: -0.52 to 0.52; $Z = 0.03$; $p = 0.97$) (Fig. 2b), and significant heterogeneity was observed ($I^2 = 98.2\%$, $p < 0.0001$).

Effect of particulate matter exposure on the mtDNAcn

Studies performed to evaluate the effect of particulate matter on the mtDNAcn are listed below: Hou et al. (2013); Wang et al. (2020); Wong et al. (2017); Xu et al. (2017); Zhang et al. (2020). Wang et al. (2020) and Zhang et al. (2020) reported the results from two different cohorts that included only men; the included participants came through populations exposed to the highest or lowest levels of ambient air pollutant exposure (PM₁₀, PM_{2.5}, NO₂, and CO). The occupational exposure to particulate matter was described in the truck drivers (Hou et al. 2013), welders (Xu et al. 2017), workers from an electric steel plant (Hou et al. 2010), and housewives (Wong et al. 2017). The pooled analysis showed an increase in the mtDNAcn in highly exposed individuals to the particulate matter. The overall estimated results were of 0.16-fold (95% CIs: −0.1 to 0.43; $Z=1.18$; $p=0.24$); the linked heterogeneity was considered moderate ($I^2=74.1\%$, $p=0.002$) (Fig. 2c).

Effect of cigarette smoking on the mtDNAcn

The effect of cigarette smoking on the mtDNAcn was evaluated by Bouhours-Nouet et al. (2005), Lee et al. (1998), Masayeva et al. (2006), and Tan et al. (2008). The increase in mtDNAcn was frequently observed in smokers. The pooled analysis showed an increase of 0.48-fold (95% CIs: 0.20–0.77; $Z=3.34$; $p=0.001$) higher in smokers than non-smokers. A low and insignificant heterogeneity was determined for the overall result ($I^2=34.9\%$, $p=0.2$) (Fig. 2d). The obtained result clearly shows the importance of including cigarette smoking as a confounder exposure source.

Subgroup analysis for exploring the causes of heterogeneity

Unfortunately, we could not perform a subgroup analysis to explore the potential causes of heterogeneity because of the few included studies for heavy metal exposure and cigarette smoking. For polycyclic aromatic hydrocarbon exposure, we performed a subgroup analysis considering the classification according to NTP/OHAT risk of bias tool for exploring the causes of heterogeneity. Nevertheless, the subgroup analysis showed similar heterogeneity $I^2=92.7\%$ and $I^2=97.9\%$ for 1st tier and 2nd tier, respectively (see supplementary data 2, Fig. 1). Similarly, subgroup analysis for particulate matter exposure showed a low and insignificant heterogeneity ($I^2=0\%$; $p=0.75$) for 2nd tier. In the 1st tier, a high and significant heterogeneity was observed ($I^2=84.4\%$; $p<0.001$) (see supplementary data 2, Fig. 2). Furthermore, a subgroup analysis considering the type of exposure (environmental or occupational) was performed; the heterogeneity for

environmental exposure was low and insignificant ($I^2=0\%$; $p=0.74$). The subgroup of occupational exposure showed high and significant heterogeneity ($I^2=84.4\%$; $p<0.001$) (see supplementary data 2, Fig. 3).

In addition, we performed a subgroup analysis considering the type of tissue used for isolating mitochondrial and genomic DNA. Heterogeneity was low and non-significant for semen ($I^2=57.6\%$; $p=0.1$), oral mucosa ($I^2=0\%$; $p=0.5$), and other somatic tissues such as placenta or lung tissue ($I^2=0\%$; $p=0.6$). However, high and significant heterogeneity was observed for isolated leukocytes ($I^2=94.2\%$; $p<0.001$), whole blood ($I^2=97.4\%$; $p<0.001$), and buffy coat ($I^2=85\%$; $p=0.01$) (see supplementary data 2, Fig. 4).

Small-study effects and publication bias

Egger's funnel plot was performed to assess the publication bias in the meta-analysis. The funnel plots showed symmetrical distribution for benzene and PAHs (Fig. 3b); the visual inspection of estimated Egger's funnel plot for heavy metals, particulate matter, and cigarette smoking revealed an asymmetric distribution (Fig. 3a, c, and d, respectively). Moreover, we performed Egger's regression test for assessing small-study effects. As shown in Table 3, the small-study effects could not be expected (p -value >0.05). However, the estimated p -value ($p=0.051$) for cigarette smoking thus provides weak evidence for the presence of small-study effects.

Trial sequential analysis

The obtained results of TSA are presented in Fig. 4. Figure 4a shows the TSA for heavy metals, Fig. 4b represents the TSA for benzene and PAHs, Fig. 4c shows the TSA for PM, and finally, Fig. 4d shows the TSA for cigarette smoking. The cumulative Z -curve does not cross the required information size in all cases. Furthermore, the cumulative Z -curve does not overpass the O'Brien-Fleming boundary lines. Accordingly, the results of TSA suggest that new studies are necessary to ensure the usefulness of mtDNAcn as a biomarker.

Risk of bias assessment

Table 4 summarizes the results from the risk of bias assessment. We employed the NTP/OHAT risk of bias tool to evaluate each included study's quality. The first considered topic was the "selection bias." Next, we assessed if the comparison groups were appropriate; many included studies reported the similarities in baseline characteristics between the analyzed groups. However, many studies that analyzed

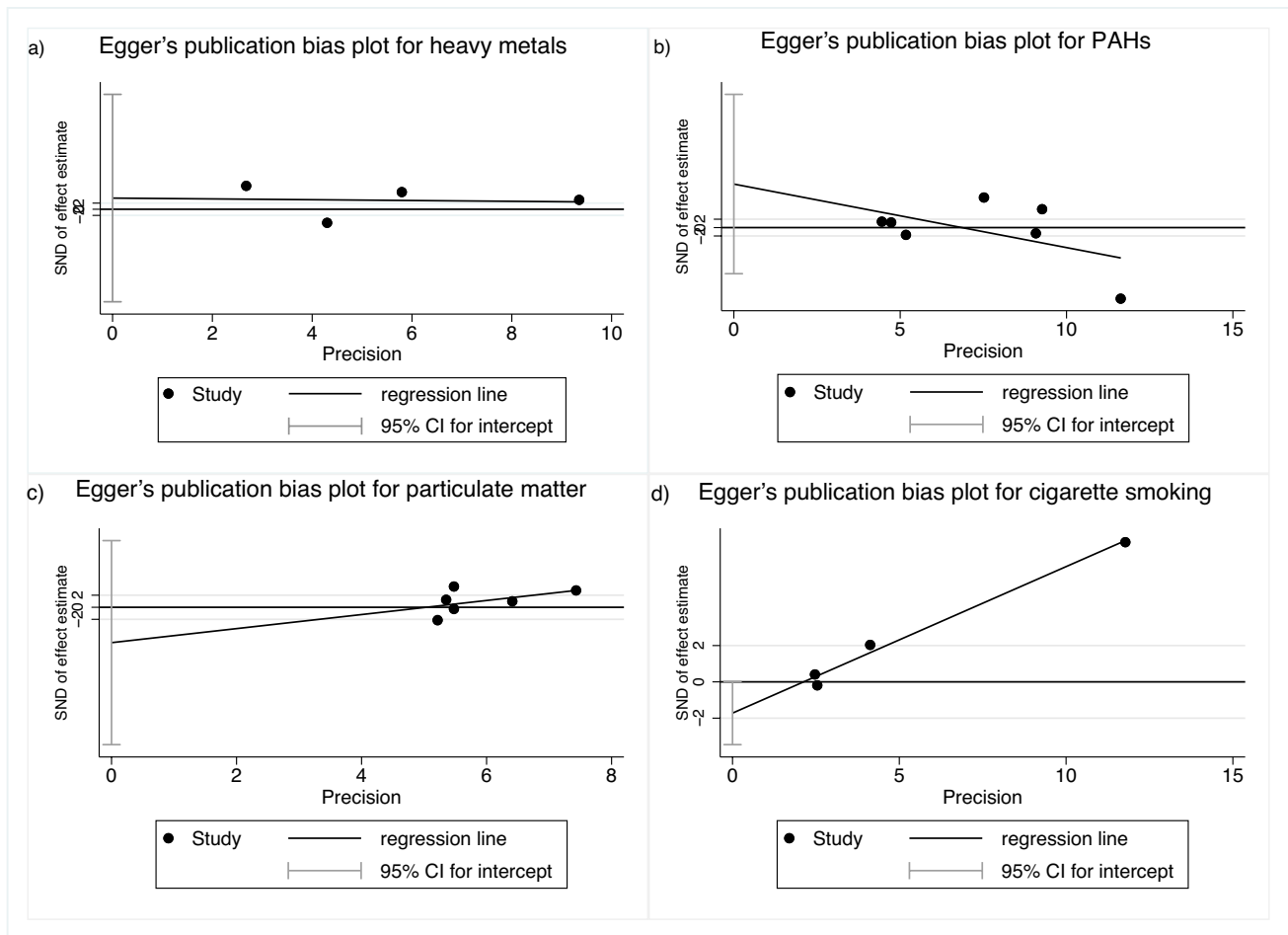


Fig. 3 Egger's funnel plot for measuring the risk of bias in studies included in the meta-analysis; circles indicate the observed standardized effect. The subsections represent the following information:

a heavy metals, **b** benzene and polycyclic aromatic hydrocarbons, **c** particulate matter, and **d** cigarette smoking

the effect of cigarette smoking on mtDNAcn did not report the similarities between the comparison groups.

The “confounding bias” topic was evaluated considering if the included studies thought the confounding and modifying variables in its design/statistical analysis. Fifty-six percent of the included studies did not adjust or control for other exposure. As previously mentioned, cigarette smoking could be considered a critical confounder. However, only one study considered cigarette smoking in the heavy metal group. For PM and PHA groups, 66% considered cigarette smoking as a confounder. A multivariate statistical approach was used to decrease the confounder effect linked to cigarette smoking in all cases. On the other hand, only 22% of the included studies considered the influence of confounding and modifying variables (key topic).

Finally, the “detection bias” topic was evaluated; a low risk of bias was considered when the included studies reported the following: the blinding in outcome analysis, the use of valid methods for measuring confounding variables,

the use of a valid method, and techniques for exposure characterization (key topic) and outcome characterization (key issue); all included studies reported the use of valid, reliable, and sensitive methods enabled to measure the changes in the mtDNAcn in response to environmental pollutant exposure. The exposure characterization was realized by quantifying the environmental pollutant or its metabolites; the studies that evaluated the effect of cigarette smoking performed the exposure assessment using standardized questionnaires. Only three records performed the outcome measuring using blinding protocols. Sixty percent of the included studies analyzed the confounding variables employing sensitive methods of assessing substantial confounding or modifying variables.

Rate confidence in body evidence

Finally, we employed the OHAT method based on the Grading of Recommendations Assessment, Development

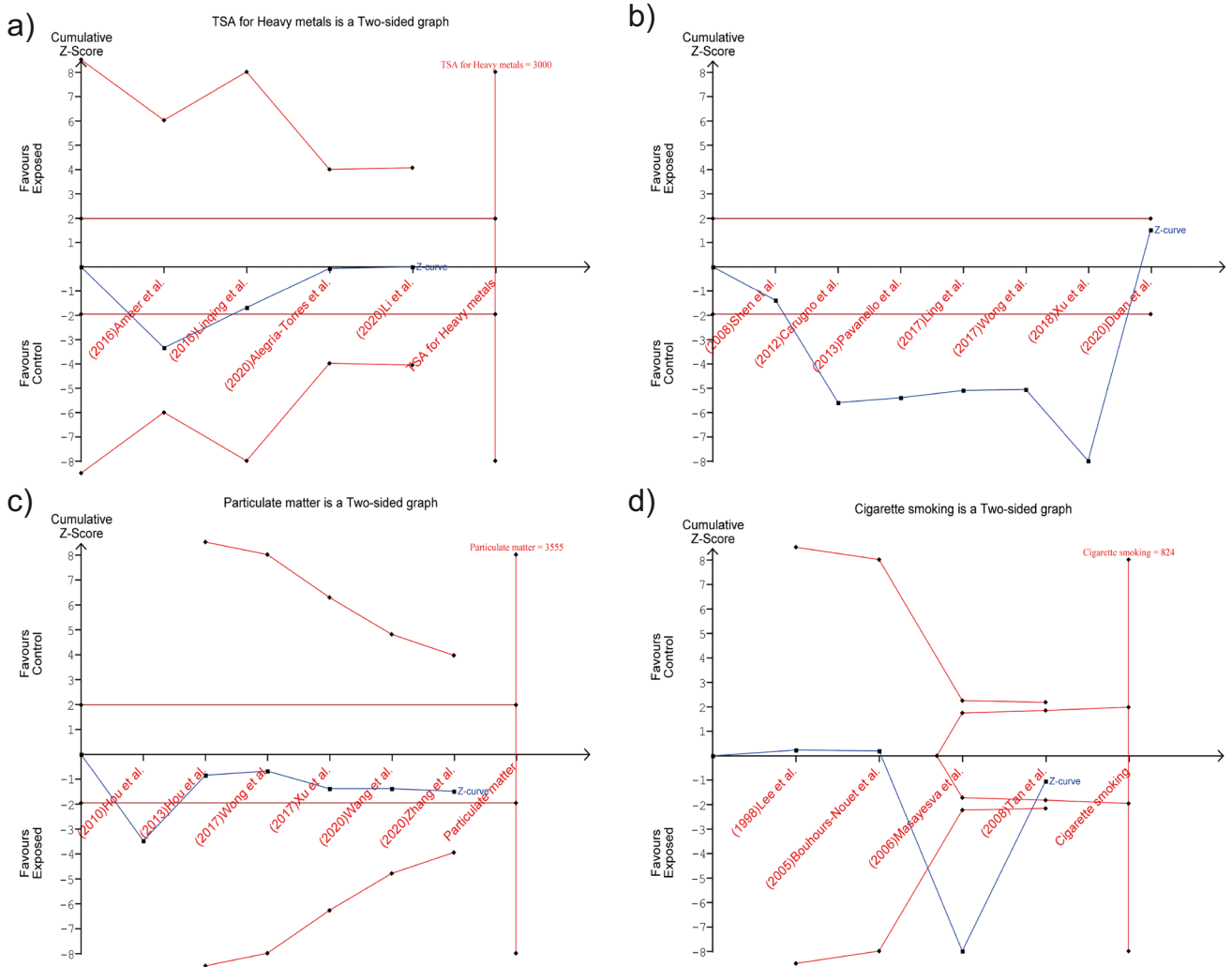


Fig. 4 Trial sequential analysis. The O’Brien-Fleming boundary line is displayed as the sloping red full lines with black diamonds, and the TSA line represents the required information size. The subsections

describe the following information: **a** heavy metals, **b** benzene and polycyclic aromatic hydrocarbons, **c** particulate matter, and **d** cigarette smoking

Table 3 Egger’s meta-regression test assessing the presence of small-study effects

Pollutant	Studies included	Participants included	Bias (intercept)	p-value	[95% Conf. intervals]
Heavy metals	4	699	3.64	0.688	− 30.10 37.37
Benzene and polycyclic aromatic hydrocarbons	9	3162	7.27	0.298	− 8.02 22.56
Particulate matter	6	1050	− 5.87	0.39	− 22.79 11.05
Cigarette smoking	4	732	− 1.72	0.051	− 3.46 0.02

and Evaluation “GRADE” to rate the confidence in the body evidence. This guide contemplates the strengths and weaknesses of the included articles; if the reported result showed strong confidence between pollutant exposure and the mtDNAcn levels, the obtained conclusion is considered high confidence. However, an unclear relationship between

exposure to environmental pollutants and mtDNAcn is considered shallow confidence. In addition, the result of rate confidence indicates directly or indirectly the need to perform new research in a particular topic. For example, suppose the body evidence is considered with high confidence. In that case, it suggests that further research is improbable

Table 4 RoB assessment for studies included in the meta-analysis

Study	Selection bias		Confounding bias		Detection bias			SUMMARY TIERED CLASSIFICATION
	Were the comparison groups appropriate?	Did the study design or analysis account for important confounding and modifying variables?	Did researchers adjust or control for other exposures that are anticipated to bias results?	Were the outcome assessors blinded to study group or exposure level?	Were confounding variables assessed consistently across groups using valid and reliable measures?	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	
Heavy metals								
Alegria-Torres et al., (2020)	+	+	-	-	+	+	++	T2
Ameer et al., (2016)	+	+	-	-	+	-	++	T2
Li et al., (2020)	+	+	-	-	-	+	+	T2
Linqing et al., (2016)	+	+	-	-	-	+	+	T2
Benzene and Polycyclic Aromatic Hydrocarbons								
Carugno et al., (2012)	+	+	+	-	+	+	+	T1
Du et al., (2020)	++	+	+	-	++	+	+	T1
Duan et al., (2020)	+	-	+	-	-	+	+	T2
Ling et al., (2017)	++	+	-	-	+	+	+	T2
Li et al., (2020)	+	+	-	-	+	+	+	T1
Pavanello et al., (2013)	+	-	-	-	-	+	++	T2
Shen et al., (2008)	+	+	-	+	+	-	+	T2
Wong et al., (2017)	+	+	+	-	+	+	++	T1
Xu et al., (2018)	+	+	+	-	+	+	+	T1
Particulate matter								
Hou et al., (2010)	+	+	+	+	+	+	++	T1
Hou et al., (2013)	+	+	+	+	+	+	++	T1
Wang et al., (2020)	+	+	-	-	-	+	+	T2
Wong et al., (2017)	+	+	+	-	+	+	++	T1
Xu et al., (2017)	+	+	+	-	+	+	+	T1
Zhang et al., (2020)	+	+	+	-	-	+	++	T2
Cigarette smoking								
Bouhours-Nouet et al., (2005)	-	--	--	--	--	-	++	T3
Lee et al., (1998)	-	-	--	-	--	+	+	T3
Masayeva et al., (2006)	++	+	-	-	-	+	+	T2
Tan et al., (2008)	-	--	--	--	--	-	+	T3

++: Definitely low risk of bias; +: Probably low risk of bias
 -: Definitely high risk of bias; -: Probably high risk of bias

to modify the confidence in the relationship between exposure to environmental pollutants and mtDNAcn. On the contrary, if the rate of body evidence is considered very low, it suggests that new research is very likely to modify the relationship between exposure to environmental pollutants and outcome.

The GRADE assessment for heavy metals, benzene and PAHs, PM, and cigarette smoking is presented in supplementary data 3 Tables 1, 2, 3, and 4, respectively. Almost included studies were performed under a cross-sectional design. For this reason, the initial rate of confidence was considered low level. Imprecision was a common downgrading factor that decreased confidence. Low confidence in the

body of evidence was the GRADE conclusion obtained for all included environmental pollutants. Larger-scale studies on this topic are required to find the role of mtDNAcn in response to environmental pollutant exposure.

Discussion

In the present study, we investigated the impact of environmental pollutants on the changes in relative levels of mtDNAcn. Although the results showed conflicting results in all included studies for each environmental pollutant, the TSA demonstrated the need to draw up and perform new studies

focusing on clarifying the relationship between environmental or occupational exposure to environmental pollutants and the changes in mtDNA. Finally, the analysis of rate confidence in body evidence showed that the available information provides weak and insufficient information.

Environmental pollution has been associated with several human diseases and exercises influence over global mortality. According to the World Health Organization, air pollution, water pollution, and poisoning are significant environmental risks (WHO 2019). Induction of oxidative stress is considered a common molecular mechanism through which environmental pollutants induce/promote/potential the development of human diseases. Classical biomarkers of oxidative stress such as antioxidant status (analyzed measuring the activity/or relative mRNA expression of key antioxidant enzymes, or total GSH level, or GSH/GSSG ratio), oxidative status (estimated by measuring of ROS levels), or oxidative damage markers (such as lipid peroxidation, carbonylated proteins or oxidative adducts in DNA) have been used for evaluating the damage induced by exposure to environmental pollutants (Ghezzi 2020; Münzel and Daiber 2018). Recently, new molecular biomarkers such as miRNAs and lncRNAs have been analyzed to identify critical patterns of expression associated with exposure to environmental pollutants (Kotsyfakis and Patelarou 2019; Miguel et al. 2020). Therefore, the changes in the mtDNA in response to environmental pollutant exposure have been analyzed with contradictory results. For this reason, we performed a systematic review and meta-analysis following the recommendations of the NTP/OHAT 2019; the NTP/OHAT 2019 indicates the guidelines for researching, selecting, extracting, synthesizing the evidence, and rating the level of confidence in the body evidence. The result could be used in environmental sciences to support state-of-science or regulatory evaluation (Rooney et al. 2014).

Exploring sources of heterogeneity

In the present meta-analysis, the standardized mean differences were performed for evaluating the changes in mtDNA according to the level of environmental pollutant exposure. Four pooled analyses ((1) heavy metals, (2) cigarette smoking, (3) benzene and polycyclic aromatic hydrocarbons, and (4) particulate matter) were performed in the present meta-analysis. Nevertheless, some of the statistical results lack statistical significance, and some suffer from excessive heterogeneity. Subgroup analysis was not performed for cigarette smoking and heavy metals because of the small number of the included studies. The minimum number of studies for performing a subgroup analysis is five studies (Spineli and Pandis 2020). Nevertheless, the heterogeneity for cigarette smoking is considered low and insignificant; low levels of heterogeneity are associated with

common variation of the included studies; the studies that analyzed the effect of cigarette smoking were performed in individuals with self-report of cigarette consumption. Moreover, almost included studies were considered with a similar risk of bias. In this line, the risk of bias estimated for each included study is regarded as a critical point directly associated with the heterogeneity. The inclusion of low-quality studies can potentially cause a low-quality conclusion. For this reason, we performed a subgroup analysis considering the final score of risk of bias according to the NTP/OHAT risk of bias tool. However, only the subgroup analysis of PM showed a reduction in heterogeneity; and surprising studies with probably low risk of bias showed low heterogeneity compared to studies with definitely low risk of bias. The assessment of environmental pollutant exposure is considered a high source of heterogeneity (Gong et al. 2022); nevertheless, the present study almost included studies that used methodologies that allow confidence in the environmental pollutant exposure. Moreover, heterogeneity in the meta-analysis is influenced by variations in individual human responses to environmental pollutant exposure. It is widely known that the variation in the outcome is associated with genetic polymorphism, age, and sex (Aldridge et al. 2003). Another point of consideration is that Egger's funnel plot for cigarette smoking, heavy metals, and PM showed an asymmetrical distribution; the asymmetric distribution indicates the lack of negative results and leads to an overestimation of pooled results (Lin 2019). However, Egger's regression shows non-evidence of small studies effects indicating that the obtained pooled results have been estimated based on studies with significant and insignificant results (Schwarzer et al. 2015).

Impact of tissue on the measuring of mtDNA

The whole blood was the most frequent biological matrix used for estimating the mtDNA because of accessible collection and relatively minimally invasive techniques. However, whole blood is a heterogeneous mix composing for 55% of blood plasma and 45% of the formed elements (approximately 98% of the formed elements are composed of erythrocytes, and the remaining 2% are covered for leukocytes and platelets together); typical results from leukocytes differential count are described below: neutrophils (50–70%), eosinophils (2–4%), basophils (0.5–1%), lymphocytes (25–45%), and monocytes (3–8%) (Marieb and Hoehn 2010). Platelets are more abundant than leukocytes (proportion 40:1); they contain mtDNA but lack nuclear DNA. Considering that the estimation of mtDNA is frequently calculated as mtDNA/nuclear DNA ratio, the number of platelets in whole blood could be used as an adjust factor from mtDNA for each studied individual (Hurtado-Roca et al. 2016). Typically, the use of buffy coat as a

biological matrix results in lower levels of mtDNAcn than whole blood; reduction is attributed to a decrease in the high influence exercise for the number of platelets in the sample (Andreu et al. 2009). Another biological matrix used for determining the levels of mtDNAcn was the isolated peripheral blood mononuclear cells (PBMCs) using density gradients. However, PBMCs and platelets show similar relative density resulting in an overestimation of mtDNAcn attributed to platelet contamination (Urata et al. 2008). Platelet elimination before PBMCs obtention decreases the risk of overestimating mtDNAcn linked to platelet contamination (Banas et al. 2004).

Rausser et al. (2021) (using fluorescence-activated cell sorting) have reported other critical issues for consideration when blood is used for measuring the mtDNAcn: (1) mtDNAcn varies according to specific cell phenotype; for example, lymphocytes B show higher levels of mtDNAcn than neutrophils; (2) mtDNAcn displayed substantial weekly variation in the same individual (changes does not attribute to genetics, constitutional, or behavior factors); (3) serum lipid levels (that including triglycerides, total cholesterol, and low- or high-density lipoproteins) were correlated with substantial changes in mtDNAcn; (4) a sexual dimorphism for mtDNAcn is observed (men showed higher mtDNAcn in comparison with women); and (5) mtDNAcn increases according to age (for each decade an increase ~ 10% is observed in monocytes and naïve T cells); the above-cited results are based in isolated PBMCs. In whole blood or buffy coat, the increase of age was negatively associated with mtDNAcn; a marked hedging effect is observed in persons older than 50 years (Mengel-From et al. 2014; Verhoeven et al. 2018). However, a recent cohort study has shown that reducing mtDNAcn attributed to age could be explicitly accounted for by the platelet count and differential percentage in leukocytes (Moore et al. 2018). Neither of the included studies (using whole blood, buffy coat, or isolated PBMCs) in the meta-analysis considered a statistical adjustment using the platelet count. Only two included studies use the total leukocyte count (Alegría-Torres et al. 2020) or percentage of neutrophils (Hou et al. 2010) as statistical adjusters. Considering the current level of evidence on isolated mtDNAcn from blood, we suggest using PBMCs isolated with a previous step of platelet elimination with statistical adjustment that includes biological sex, neutrophil/lymphocyte ratio, age, and serum lipid levels (triglycerides and total cholesterol) for future studies. For example, suppose the authors used whole blood or buffy coat. In that case, they could include a statistical adjustment by biological sex, platelet count, differential percentage in leukocytes, age, and serum lipid levels (triglycerides and total cholesterol).

Other constraints to consider include that mtDNAcn isolated from blood could not always reflect a genuine change in mtDNAcn for another tissue or organ. Correlation analysis

of mtDNAcn from different human tissues (that including blood, cerebellum, cerebrum, cortex, kidney, large intestine, liver, myocardial muscle, ovary, small intestine, skin, and skeletal muscle) showed that the mtDNAcn level from blood does not correlate with measured mtDNAcn levels for another organ/tissue (Wachsmuth et al. 2016). While most xenobiotics cross several barriers from the point of contact to target tissue/organ, a bloodstream step is required. For this reason, the toxicokinetic associated with a particular environmental pollutant could be considered before selecting the blood as an excellent biological matrix for the isolation of mtDNA.

Semen, oral mucosa, and somatic tissues (lung and placenta) were other tissues used to measure the mtDNAcn in the included studies. At the same time, a low and insignificant heterogeneity was linked to the use of these tissues. There are some issues to consider. As is well known, the number of mitochondria changes in accordance with tissue-specific energetic demands directly linked to the rate of oxidative phosphorylation activity. For example, the muscle, heart, and liver showed higher levels of mitochondria than the kidney and lung (D'Erchia et al. 2015). The use of lung tissue could be associated with low levels of mtDNAcn and marginal changes linked to environmental pollution exposure. Furthermore, the use of semen for measuring mtDNAcn is limited because mammalian spermatozooids are characterized by a low number of mitochondria (22 to 75) with low mtDNAcn (for example, human spermatozoa show a range of 2.8 to 226 mtDNAcn for each spermatozoon) (Song and Lewis 2008). In addition, semen contains ~15% of nonspermatozoal cells (including immature germ cells, epithelial cells, and leukocytes) (Fedder 1996), which could cause a wrong estimation of real changes in spermatic mtDNAcn associated with environmental pollutant exposure. The selection of mitochondrial gen for measuring mtDNAcn in spermatozooids is another crucial topic. A study performed in isolated spermatozooids from horse semen showed that the use of cytochrome b (cytb) and ND4 (mitochondrially encoded NADH dehydrogenase 4) genes results in the overestimation of mtDNAcn in comparison with the use of ND1 (mitochondrially encoded NADH dehydrogenase 1) gen. The authors suggested that the localization of cytb and ND4 into mtDNA (near zones of origin of heavy strands replication and the origin of light strand replication) results in a high deletion rate of cytb and ND4. Furthermore, this fact is associated with an overestimation of mtDNAcn (Darr et al. 2017). We suggest using isolated spermatozooids to measure the impact of environmental pollutant exposure on spermatic mtDNAcn. If the authors use semen, we suggest performing a count of nonspermatozoal cells for statistical adjustment.

Moreover, the number of spermatozoa in both sceneries could be used as another statistical adjuster. There is no evidence of changes on mtDNAcn according to the class of

epithelium cells (keratinocyte differentiation). However, an experimental study showed that the number and dimension of mitochondria decrease in the upper cell layer (Simpson et al. 2021). For this reason, the loss of mitochondria (from basal cells to differentiated cells) and the loss of cell nuclei (from differentiated cells to karyolytic cells) through the differentiation cycle of epithelial cells could be considered when the stratified squamous epithelium from oral mucosa is used for measuring the mtDNA_{cn}.

The potential use of mtDNA_{cn} as pollution biomarker

It is widely accepted that the ideal biomarker for characterizing the environmental pollution exposure would potentially have the following characteristics: (1) the use of biomarker has no ethical issues; (2) the obtention and analysis of biomarker are distinguished primarily by its minimal invasion and reliable results; (3) the biomarker is specific for a particular pollutant; (4) the changes in biomarker levels reflect reversible subclinical/clinical effects and predict or monitor patient response to intervention/treatment medical (Gil and Pla 2001). Therefore, mtDNA_{cn} has shown an easy obtention without ethical implications. The mtDNA_{cn} measurements use the latest technology and instruments (that include quantitative real-time PCR: qPCR; digital PCR: dPCR, and genotyping microarray probe intensities and DNA sequencing read counts such as whole-exome sequencing: WES, and whole-genome sequencing: WGS). Comparison of these techniques showed that WGS could be considered the gold standard for measuring the mtDNA_{cn} compared to the current gold standard qPCR and dPCR. Furthermore, the authors showed a significant variation in mtDNA_{cn} levels associated with the employed method for isolating DNA (Longchamps et al. 2020). The changes in mtDNA_{cn} are not environmental pollutant-specific; several environmental pollutants induce change in the mtDNA_{cn} (see supplementary table 5 for visualizing other studies). Moreover, the association between the changes in mtDNA_{cn} levels and clinical effects has yet to be clarified. For example, an increase in mtDNA_{cn} has been associated with the development of cardiovascular diseases (Ashar et al. 2017), and leukemia, myeloproliferative, and spleen diseases (Hägg et al. 2021), while neurodegenerative diseases are associated with a reduction in mtDNA_{cn} (Yang et al. 2021). Changes in birth weight and cognitive/locomotor development are frequent clinical events studied about changes in placental mtDNA_{cn} induced by air pollution; nevertheless, contradictory results are reported through published reports (Li et al. 2019). Another studied clinical event is the changes in blood pressure in response to air pollution

and its association with the mtDNA_{cn}. The reported results showed that subjects exposed to similar levels of air pollution showed a differential increment in blood pressure associated with mtDNA_{cn} levels. Subjects with the highest mtDNA_{cn} showed a minor increase in blood pressure (in response to air pollution) in comparison with the lowest mtDNA_{cn} subjects (Zhong et al. 2016). Unfortunately, the currently available information is not able to state the facts between a clinic event and the changes in mtDNA_{cn} induced by the exposure to environmental pollutants in a clear manner. Moreover, the biological effect of modifications in mtDNA_{cn} levels is unclear. Early reports suggested that an increase in the mtDNA_{cn} could be an indicator of mitochondrial biogenesis and/or a mechanism for compensating defects in the functionality of mitochondrial complexes that results in a decrease from the bioenergetic capacity of mitochondrial (Miyako et al. 1997; Renis et al. 1989). However, correlation analysis between mtDNA_{cn} and mitochondrial content in skeletal muscle from healthy young subjects showed that mtDNA_{cn} levels are not linked to mitochondrial content (Larsen et al. 2012). A similar result showed that VDAC1 levels (a biomarker of mitochondrial mass) are not correlated to mtDNA_{cn} levels in human skeletal muscle and hippocampus (Brinckmann et al. 2010). In addition, the increase of mtDNA_{cn} by transfection of transcriptional mtDNA factors (such as TFAM or Twinkle) in mice does not change the levels of mitochondrial complexes (biomarkers of mitochondrial activity) (Ikeda et al. 2015). For these reasons, an increase of mtDNA_{cn} could not be considered a good standard to indirectly measure the mitochondrial status of biogenesis, content, or bioenergetic activity. Conversely, a reduction of mtDNA_{cn} could be a positive indicator of mitochondria's bioenergetic adaptation or biogenesis. For example, an increase in activity/relative mRNA levels and protein content from citrate synthase, cytochrome c oxidase subunit IV, and cytochrome c (biomarkers of bioenergetic functionality of mitochondrial) was observed in human skeletal muscle from subjects under short-term, aerobic exercise training. But mitochondrial fission regulator DRP-1 (dynamamin-related protein 1) and mitochondrial fusion regulator Mfn (mitofusins) showed an increase in the protein content and relative mRNA levels, respectively. Surprisingly, a reduction in mtDNA_{cn} levels was observed (Egan et al. 2013). Isolated human fibroblasts exposed to cigarette smoke condensate (at 80 µg/mL by 72 h) showed an increase in biomarkers of mitochondrial mass and biogenesis such as citrate synthase, the subunit A of succinate dehydrogenase, and nuclear respiratory factor 1; nevertheless, the mtDNA_{cn} levels were reduced (Giordano et al. 2015). As can be seen, there is a need for performing new studies that show the molecular, cellular,

biochemical, and biological repercussions linked to change in mtDNAcn levels.

A possible causal link between environmental pollutant exposure, oxidative stress, and mtDNAcn

Mitochondria are considered a subcellular target for many environmental pollutants; while the exact mechanism of environmental pollutant-induced mitochondrial toxicity is not fully known, it is considered to occur either as a consequence of the oxidative stress induction that results in mitochondrial dysfunction (Meyer et al. 2018). ROS regulates several cellular signaling pathways in normal physiological conditions, including nuclear-mitochondrial communication (Moldogazieva et al. 2018). It has been observed that under an oxidative stress state, the mtDNAcn increases in a dose-time-dependent manner (Lee et al. 2000). This fact could be explicated by activating the Keap1-Nrf2 pathway; the increase in cytoplasmic ROS promotes the oxidation of Keap1, abolishing its repressive function over Nrf2. Then, Nrf2 is translocated to the nucleus and promotes transcription of antioxidant response element and other genes (Deshmukh et al. 2017). Both nuclear transcriptional factors Nrf2 and Nrf1 are implicating in the biogenomic coordination between nuclear and mitochondrial genomes and the regulation of mitochondrial biogenesis (Goffart and Wiesner 2003). Moreover, the mtDNAcn levels could be regulated by Nrf1 and Nrf2; both factors can recognize consensus-binding sites in mitochondrial transcription factor A (mtTFA) (Virbasius and Scarpulla 1994); mtTFA has been directly linked to regulation of the mtDNAcn in mammalian cells (Ekstrand et al. 2004). Considering that several environmental pollutants can induce an overactivation of transcriptional factors, including the Nrf1 and Nrf2 (Jennings et al. 2013; Venugopal and Jaiswal 1998), it is possible that the increase in mtDNAcn could be considered the result of environmental pollutant-induced activation of Nrf1 and Nrf2. However, more mechanistic studies are required to verify this proposed theory.

Environmental pollutant's affinity for mtDNA

Another point to consider is the significant variation in the affinity to mtDNA over nuclear DNA. For example, early reports showed that PAHs have a high affinity to produce adducts in mtDNA in comparison with nuclear DNA (Allen and Coombs 1980); it results in a large adducts formation in mtDNA approximately 40- to 90-fold higher in comparison with nuclear DNA (Backer and Weinstein 1980); on the other hand, a differential affinity for mtDNA or nuclear DNA has not been proven for heavy metals; nevertheless, these are associated with the induction of oxidative stress and the formation of oxidative adducts in DNA (8-OHdG mainly),

and decrease the expression of key enzymes involved in DNA repair mechanism (OGG1 mainly) (Al Bakheet et al. 2013); similar mechanisms are linked to particulate matter exposure; the exposure of human endothelial cells to PM results in an increase in mitochondrial ROS, and the disrupt of key mitochondrial functions such as regulation of both mitochondrial biogenesis and fission (Wang et al. 2020). These facts could explain the non-systematic effect of environmental pollutant exposure on mtDNAcn.

Limitations

In the present work, we analyzed the effect of exposure to environmental pollutants in changes on mtDNAcn levels. We performed a comprehensive search strategy in the most appropriate databases. And the literature selection, risk of bias evaluation, and rate confidence in the body of evidence were performed employing the OHAT's tools because these tools were developed to be used in systematic review and meta-analysis with environmental epidemiology implications. There are limitations associated with our study: (1) the obtained result derivatives of subgroups that contain heterogeneous environmental pollutants; (2) some included studies do not perform a complete characterization of exposure to environmental pollutants; (3) most included studies were performed using a cross-sectional design that increases the risk of bias; (4) the obtained results do not represent a dose-response effect because the pooled analysis was carried out considered only the lowest level of exposure versus highest level. The mentioned limitations should be considered when interpreting the obtained results.

Conclusions

The systematic review and meta-analysis show evidence that exposure to environmental pollutants induces marginal changes on mtDNAcn levels. Nevertheless, the data obtained in this study also suggest the need to perform new studies with mechanistic or cohort designs are required to elucidate the biological effect associated with the changes in mtDNAcn. And its possible use as a biomarker linked to environmental pollution exposure.

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Author contribution Conceptualization: Marco Antonio Ramírez-Vargas. Investigation: Marco Antonio Ramírez-Vargas and Cristian Avilés-Ramírez. Formal analysis: Eugenia Flores-Alfaro and Mónica Ramírez. Visualization: Cristian Avilés-Ramírez and Gerardo Huerta-Beristain. Writing—original draft: Ma. Elena Moreno-Godínez and Marco Antonio Ramírez-Vargas. Writing—review and editing: Isela Parra-Rojas and Matthew R. Bonner.

Data availability All the data and material pertinent to this manuscript are included and have been reviewed by all authors.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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