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RESEARCH ARTICLE

Arsenic and subclinical vascular damage in a sample of Italian young adults: a cross-sectional analysis

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Abstract Exposure to arsenic (As) increases cardiovascular risk. The purpose of this study was to evaluate the relationship between As and intima-media thickness (IMT) in the common carotid artery and common genetic variants in genes implicated in As metabolism (ASIIIMT Met287Thr, GSTT1+/−, and GSTM1+/−) and DNA repair (hOGG1 Ser326Cys and XRCC1 Arg399Ser). Two hundred and fourteen healthy volunteers, age 20–46, were recruited in four zones polluted by As. Urine samples were tested for total As, inorganic As (iAs), monomethylarsinic (MMA), and dimethylarsinic acid (DMA). Primary and secondary methylation index (PMI, SMI) were computed as MMA/iAs and DMA/MMA. Common carotid artery scans were obtained by high-resolution ultrasound. There was no correlation between IMT and total As, iAs, iAs + MMA + DMA, PMI, or SMI. However, the increase of IMT with age was higher than that observed in the healthy population, both in males (6.25 vs. 5.20 μm/year) and, to a lesser extent, in females (5.05 vs. 4.97 μm/year). After correction for age and gender, subjects with a high urinary As level (≥3.86 μg/L) and carriers of the GSTT1-positive (+) genotype also had higher IMT than those with a low urinary level and the GSTT1-null (−) genotype (0.56 [0.48–0.64] vs. 0.53 [0.44–0.62] mm, $p = 0.010$). The analysis hints at faster vascular aging as compared to the healthy population. Our findings also suggested that GSTT1 and hOGG1 gene polymorphisms might play an important role in the individual risk of As-induced carotid atherosclerosis.

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Introduction

Arsenic (As) is a natural element of the earth's crust whose toxicity has been known for centuries. Adverse effects depend on dose, duration, and frequency of exposure (Abernathy et al. 1999) and can range from neurological and respiratory damage to the onset of cancers (WHO-IARC 2012; Leonardi et al. 2012; Garcia-Esquinas et al. 2013; Steinmaus et al. 2014; Stea et al. 2014; Tyler and Allan 2014). The main source of chronic intoxication for the general population is ingestion of water drilled in contaminated deposits, occurring mainly in developing countries and also, to a lesser extent, in the Western world: the World Health Organization has set a limit of 10 μg/L for drinking water (WHO-IARC 2012). Several population studies relate exposure to high levels of As to an increased incidence of ischemic heart disease and cardiovascular (CV) mortality (Stea et al. 2014).

The association between exposure to high levels of As, CV risk factors such as hypertension and diabetes mellitus, and vascular damage such as carotid atherosclerosis is established (Stea et al. 2014); less explored are the effects of lower levels (Moon et al. 2013; Tsuji et al. 2014; Farzan et al. 2015; Wade et al. 2015; Sidhu et al. 2015; Mendez et al. 2016). Specifically, moderate As exposure was recently associated with several markers of increased cardiometabolic risk (diabetes, triglyceridemia, and cholesterolemia) in Mexico's population (Mendez et al. 2016). A case-control study in Inner Mongolia, China, reported that the risk of CV disease among subjects with drinking water arsenic above 40 μg/L was four times higher compared to those with drinking water arsenic

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less than 10 μg/L. A 10 μg/L increase in water arsenic was associated with a 19 % increased risk of CV disease (Wade et al. 2015). Interestingly, Farzan et al. (2015) also showed in a US population-based study a synergistic relationship between arsenic exposure and smoking on health outcomes supporting a role for lower-level exposure in ischemic heart disease mortality.

Moreover, outcome measures with their potential confounders may have played a role in the non-significance of many statistical associations (Tsuji et al. 2014; Sidhu et al. 2015; Ameer et al. 2015). Nonetheless, on a global scale, even a modest increase in risk might have substantial impact on morbidity and mortality. Although mechanisms through which As can cause atherosclerotic lesions and their complications have been explored, individual risk from environmental pollution remains to be established (Stea et al. 2014). Additionally, there is a high inter-individual variability in the susceptibility to As toxicity, and this is thought to be related to the presence of specific gene polymorphisms (Faita et al. 2013). Studying individual susceptibility through genetic testing and imaging biomarkers of atherosclerosis could shed a light on the real impact of low-dose exposure and individual CV risk (Faita et al. 2013).

Therefore, the purpose of this study was to evaluate the relationship between As and intima-media thickness (IMT) in the common carotid artery, a known marker of subclinical vessel damage and a predictor of CV accidents (Lorenz et al. 2007), as well as the presence of common genetic variants in genes implicated in As metabolism (ASIIIMT Met287Thr, GSTT1+/−, and GSTM1+/−) and DNA repair (hOGG1 Ser326Cys and XRCC1 Arg399Ser) (Faita et al. 2013).

Materials and methods

Study population

We studied 229 volunteers aged 20 to 46 who participated in the SepiAs project (Sorveglianza epidemiologica in aree interessate da inquinamento ambientale da arsenico) whose rationale has been previously published (Bustaffa and Bianchi 2014). The participants were recruited through advertisement in local media and around cities and villages in four zones of Italy known to be affected by As pollution: Viterbo (2 municipalities) and Mount Amiata (1 municipality), where As from natural sources contaminates water, and Taranto (1 municipality) and Gela (2 municipalities), affected by heavy industrial pollution originating from large plants and factories within the city limits, including As compounds in fumes and waste. The basic criterion to enter the study was having been living in the area for 6 months at least. All the four referring health centers were requested to recruit at least 25 male and 25 female subjects. Participants were administered a questionnaire on life habits, work, medical history, and diet in a face-to-face interview.

As assay

Each subject collected a sample of first-urine void in a test tube and was asked to keep it in the house refrigerator until carried to the laboratory as early as possible in the same morning. Then, the urine was frozen and kept at −30 °C until the analysis.

The sample was tested for inorganic As (iAs; including trivalent and pentavalent), for methylated metabolites, i.e., monomethylarsinic and dimethylarsinic acid—MMA (V) and DMA (V), respectively, and for total As, calculated as the sum of iAs, methylated metabolites, and other forms of arsenic (e.g., arsenobetaine, arsenocholine).

Quantification was performed by separation through high-performance liquid chromatography (HPLC) and mass spectrometry. Samples were defrosted and then diluted 1:2 with bi-distilled water and filtered. Fifty microliters was injected in the anion-exchange column (Dionex AS9 SC 250 mm × 4 mm) of the chromatograph (Binary IC pump series 250—PerkinElmer). Fractions were diluted in 1 % HNO_3 and analyzed with inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS; ELAN DRC II, PerkinElmer Sciex Instrument, Toronto, Canada).

Primary methylation index (PMI) was defined as MMA/iAs and secondary methylation index (SMI) as DMA/MMA (Huang et al. 2009). Subjects were requested not to eat fish or seafood in the 3 days before the analysis.

Carotid ultrasound

Ultrasound scans of the common carotid on both sides were acquired by a trained cardiologist with the locally available commercial device (Biosound Esaote MyLab 30; Hewlett-Packard SONOS 7500; Philips iE33) equipped with a 7-MHz linear probe, aiming at obtaining the best possible image with the vessel in the horizontal position and a clear vessel-lumen interface. Twenty-five-frames-per-second video clips were recorded, about 10 s long. All clips were analyzed off-line in a single center—Pisa—by a trained operator using an automatic edge-detection software (Cardiovascular Suite, Quipu Srl, Pisa, Italy) (Faita et al. 2008). IMT and diameter considered for analysis are the mean value from the two sides.

Genotyping assay

Genomic DNA was extracted from peripheral blood leukocytes. GSTM1 and GSTT1 genotypes were determined using a co-amplification polymerase chain reaction (PCR) approach with the GSTM4 gene, which is never deleted, as internal control in order to distinguish the null genotypes from aborted

PCR (Zhong et al. 1993). Primer sequences, annealing temperatures, and digest conditions were performed according to our previously published protocols (Manfredi et al. 2009). The internal standard fragment amplified from the GSTM4 gene was 157 bp. A 230-bp fragment was amplified for the GSTM1 gene, and a 480-bp fragment was obtained for the GSTT1 gene. The absence of amplified products was consistent with the null genotypes. ASIIIMT Met287Thr, hOGG1 Ser326Cys, and XRCC1 Arg399Ser polymorphisms were analyzed by PCR-RFLP analysis (Andreassi et al. 2009). Briefly, PCR products were digested with specific restriction enzymes that recognized and cut either the wild-type or the variant sequence site. The digested PCR products were analyzed on 10 % polyacrylamide gels and stained with ethidium bromide. Genotype results were regularly confirmed by random repetition of the samples. All uncertain results were reanalyzed with the same technique, and usually one more assay was sufficient to clarify any doubts.

Statistical analysis

Data are expressed as median [25–75 %] or categories. Published normal values of IMT for the general healthy population were taken as a reference (Engelen et al. 2013). Correlation between two sets of variables was assessed accordingly through Spearman's ρ; differences between groups were tested with Mann-Whitney *U*. The Kruskal-Wallis test was also used to compare the differences in As/metabolite levels and IMT values among three different groups. Each genotype was assessed according to dominant (wild-type homozygote vs. heterozygote and homozygote variant), recessive (wild-type homozygote and heterozygote variant vs. homozygote variant), and additive (wild-type homozygote vs. heterozygote variant vs. homozygote variant) genetic models. A two-way ANOVA was used to examine the interaction between the genotypes and exposure on IMT after controlling for other confounders. A probability $p < 0.05$ was deemed significant; Bonferroni correction was applied to all multiple comparisons.

Results

Of the 229 subjects enrolled, 214 urinalysis and suitable scans of the common carotid were available. Sex and age distribution, together with smoking habit, diabetes, dyslipidemia, hypertension urinary As speciation, and results from the carotid scan are shown in Table 1.

The proportion of subjects with CV risk factors on the overall population was very small; therefore, these were not included as potential confounders in data analysis. No subject had IMT above the limit commonly set to define subclinical vascular damage (0.90 mm) (Mancia et al. 2013). Ten of the subjects (4.7 %) had IMT over the 97.5th percentile for their age (Engelen et al. 2013): this subgroup did not differ from the rest of the sample, neither for As levels nor methylation indexes (p = ns). Two (0.9 %) had IMT lower than the 2.5th percentile for their age.

As expected, IMT was correlated with age ($\rho = 0.50$, $p < 0.001$) and carotid diameter ($\rho = 0.49$, $p < 0.001$). Smokers did not have a significantly higher IMT ($p = 0.25$). There was no correlation between carotid IMT and total As ($\rho = 0.02$, $p = 0.83$), iAs ($\rho = 0.02$, $p = 0.83$), the sum of iAs + MMA + DMA ($\rho = 0.01$, $p = 0.85$), PMI ($\rho = -0.04$, $p = 0.57$), or SMI ($\rho = 0.05$, $p = 0.46$), neither in the overall population nor analyzing each area separately (all p = ns).

No effect of As on IMT was observed neither when age, diameter, smoking status, and sex were introduced in a multivariate linear regression model (standardized beta for iAs + MMA + DMA = 0.03, $p = 0.59$; age = 0.44, $p < 0.001$; diameter = 0.35, $p < 0.001$; smoke = 0.03, $p = 0.62$; sex = −0.01, $p = 0.90$; adjusted R^2 for the model =0.34, $p < 0.001$).

In order to evaluate a possible threshold effect for an evidence of effect of As on IMT, the analyses were repeated dichotomizing the population according to the suggested threshold for iAs (≥3.86 μg/L) (Hays et al. 2010; Borghini et al. 2016). No correlation with IMT was found below ($\rho = -0.04$, $p = 0.6$) or above ($\rho = 0.2$, $p = 0.1$) the levels of iAs. Additionally, when stratified by both natural and artificial pollution, no significant differences were observed.

However, the increase of IMT with age was apparently higher than that observed in the healthy reference population (Engelen et al. 2013), both in males (6.25 vs. 5.20 μm/year) and, to a lesser extent, in females (5.05 vs. 4.97 μm/year), as shown in Fig. 1. The constant in the linear equation (i.e., the intercept value) is 354.1 vs. 323.5 of the reference population for males, 337.5 vs. 321.7 for females, so study individuals also seem to have a higher IMT than the reference to begin with.

Genetic results showed significant associations between GSTT1 and PMI ($p < 0.05$, Table 2). Carriers of AS3MT Met287Thr polymorphism tended to have a higher PMI, although not statistically significant ($p = 0.068$, Table 2). DNA repair (XRCC1 Arg399Ser and Ser326Cys) polymorphisms were not related to any of the indicators of the As exposure and metabolic capacity (Table 2).

The combined effects of As levels with genetic variants on IMT were also evaluated. Subjects with a high urinary As level (≥3.86 μg/L) and carriers of the GSTT1-positive (+) genotype also had higher IMT values than those with a low urinary level and the GSTT1-null (−) genotype (0.56 [0.48–0.64] vs. 0.53 [0.44–0.62] mm; $F = 6.1$, $p_{interaction} = 0.010$) (Fig. 2). Additionally, subjects with a high urinary As level (iAs ≥ 3.86 μg/L) and hOGG1 Cys allele had significantly higher IMT (still within normal values) than those with a low urinary level and hOGG1 Ser-Ser genotype (0.58 [0.52–

Table 1 Subjects, classical risk factors, As speciation, and carotid parameters in the four centers

	Subjects (M/F)	Age	Smoke (Y/N)	Diabetes (Y/N)	Dyslipidemia (Y/N)	Hypertension (Y/N)	Total As	iAs
Amiata	29 (13/16)	33.0 [25.8–38.0]	9/20	0/29	1/28	2/27	5.72 [3.44–10.00]	0.80 [0.28–1.09]
Gela	81 (42/39)	33.4 [24.9–40.5]	22/59	1/80	1/80	2/79	20.69 [9.83–59.8]	2.31 [1.18–14.23]
Taranto	40 (17/23)	32.5 [27.0–37.5]	10/30	0/40	0/40	2/38	17.00 [9.96–43.89]	1.65 [1.24–10.21]
Viterbo	64 (30/34)	33.1 [26.5–39.1]	29/35	0/64	0/64	4/60	10.98 [6.19–19.41]	1.37 [1.13–1.80]
Total	214 (102/112)	33.0 [26.3–39.2]	70/144	1/213	2/212	10/204	13.72 [6.98–32.29]	1.57 [0.96–6.49]

	MMA	DMA	Other forms	PMI	SMI	Diameter	IMT
Amiata	0.77 [0.38–2.00]	1.37 [0.80–3.77]	3.09 [1.76–6.31]	1.20 [0.82–1.47]	2.03 [1.54–2.95]	6.75 [6.37–7.10]	0.55 [0.49–0.59]
Gela	1.81 [1.11–4.00]	5.70 [1.84–24.25]	8.73 [3.93–15.59]	0.75 [0.47–1.20]	2.81 [1.52–5.13]	6.68 [6.37–7.13]	0.51 [0.48–0.58]
Taranto	2.22 [1.70–3.29]	6.90 [4.17–11.75]	5.85 [3.25–12.70]	1.15 [0.48–1.43]	2.90 [2.48–3.35]	6.48 [6.15–6.72]	0.51 [0.47–0.55]
Viterbo	1.21 [0.89–2.08]	3.89 [2.95–6.50]	3.81 [1.33–8.30]	0.80 [0.58–1.05]	3.08 [2.40–3.56]	6.68 [6.48–7.16]	0.52 [0.46–0.56]
Total	1.75 [0.90–2.89]	4.70 [1.91–8.07]	5.42 [2.74–11.45]	0.85 [0.53–1.29]	2.90 [1.92–3.59]	6.62 [6.36–7.05]	0.51 [0.48–0.57]

Values are median [25–75 %]

iAs inorganic As, *MMA* monomethylarsinic acid, *DMA* dimethylarsinic acid, *PMI* primary methylation index (MMA/iAs), *SMI* secondary methylation index (DMA/MMA), *IMT*, *common* carotid intima-media thickness

0.65] vs. 0.52 [0.47–0.57] mm; $F = 8.2$, $p_{\text{interaction}} = 0.010$) (Fig. 3).

Discussion

This cross-sectional analysis found no correlation between different species of urinary As and common carotid IMT in a sample of young volunteers from different areas in Italy with potential contamination by As in the environment. The thickness of the intima-media layer in the present sample was small and mostly in the normal range for the healthy population (Engelen et al. 2013). Nevertheless, the study seems to support the hypothesis of an accelerated vascular aging compared to the general healthy population.

Additionally, our data showed statistically significant interactions between urinary As levels and GSTT1 and hOGG1 polymorphism suggesting that combined analyses of imaging and genetic biomarkers may be useful to better define the risk profiling of a sample of healthy and young volunteers.

Chronic exposure to relatively low doses, especially through contamination of drinking water, is a global problem, mainly, but not exclusively, in the developing world. The main concern and the best known effect is cancerogenicity, but human exposure is also associated with atherosclerosis and increased CV disease (Stea et al. 2014).

Natural contamination of underground water in Italy—although at lower levels than those found in countries such as Taiwan or Bangladesh—occurs in some areas such as Mount Amiata or the Viterbo Province, so that in some parts of the

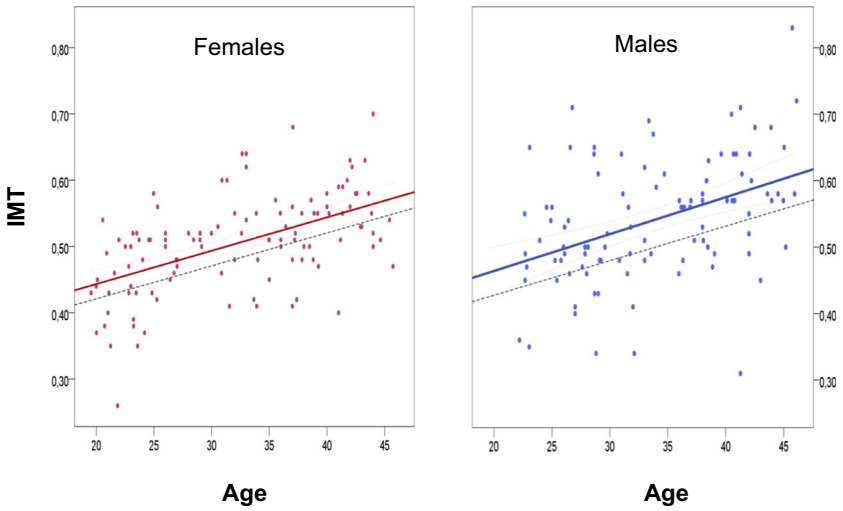

Fig. 1 IMT tended to have a steeper correlation with age than that of the healthy reference population (Engelen et al. 2013)

Table 2 Genotypes and As speciation

Genotypes	Total As	iAs	iAs + MMA + DMA	PMI	SMI
ASIIIMT Met287Thr					
MetMet (n = 145)	12.68 [6.89–26.97]	1.49 [0.97–3.47]	7.44 [3.90–13.79]	0.819 [0.533–1.251]	2.81 [1.93–3.58]
MetThr (n = 63)	16.63 [8.41–43.3]	1.70 [1.01–10.67]	9.57 [4.62–28.21]	0.838 [0.359–1.278]	2.98 [1.98–4.07]
ThrThr (n = 4)	14.41 [8.46–23.31]	1.55 [0.92–1.67]	9.53 [5.06–11.77]	1.528 [1.219–1.726]	2.78 [1.68–3.24]
GSTM1 (+/−)					
GSTM1+ (n = 84)	15.41 [7.15–38.65]	1.62 [1.02–8.58]	8.80 [4.49–26.93]	0.795 [0.531–1.194]	2.91 [1.67–3.65]
GSTM1− (n = 128)	13.14 [7.02–26.49]	1.57 [0.96–2.71]	7.83 [3.92–12.79]	0.924 [0.523–1.312]	2.88 [2.13–3.44]
GSTT1(+/−)					
GSTT1+ (n = 167)	13.43 [7.13–25.54]	1.54 [1.01–2.81]	7.82 [4.16–12.68]	0.885 [0.578–1.287]*	2.81 [1.92–3.41]
GSTT1− (n = 45)	20.53 [6.66–68.70]	1.68 [0.92–18.45]	11.16 [4.13–44.48]	0.615 [0.280–1.185]*	3.27 [1.98–5.27]
XRCC1 Arg399Gln					
ArgArg (n = 84)	14.69 [7.34–34.86]	1.60 [0.95–7.00]	8.16 [4.44–23.95]	0.806 [0.523–1.300]	3.08 [2.25–3.73]
ArgGln (n = 102)	14.20 [7.09–30.76]	1.51 [0.97–5.66]	8.38 [4.20–18.98]	0.861 [0.527–1.324]	2.70 [1.65–3.35]
GlnGln (n = 26)	11.67 [6.41–42.95]	1.58 [1.20–8.52]	7.30 [3.80–33.85]	0.889 [0.555–1.194]	2.72 [1.64–3.26]
hOGG1 Ser326Cys					
SerSer (n = 126)	14.07 [7.74–38.55]	1.59 [0.94–8.47]	8.24 [4.83–26.77]	0.824 [0.509–1.284]	2.92 [2.00–3.67]
SerCys (n = 80)	14.55 [6.73–25.30]	1.56 [1.02–2.68]	7.72 [3.70–13.56]	0.869 [0.569–1.301]	2.90 [1.90–3.35]
CysCys (n = 6)	7.70 [4.16–117.03]	1.29 [0.75–19.17]	4.37 [2.72–68.79]	0.910 [0.455–1.225]	2.38 [1.36–4.35]

Values are median [25–75 %]

iAs inorganic As, *MMA* monomethylarsinic acid, *DMA* dimethylarsinic acid, *PMI* primary methylation index (MMA/iAs), *SMI* secondary methylation index (DMA/MMA)

*$p < 0.05$ between genetic variants

latter, even the drinking water supply operated above the WHO threshold of 10 μg/L (WHO-IARC 2012). Other cities in the country still endure heavy industrial pollution, such as Gela in Sicily, mainly due to petrochemical plants, and Taranto in the southeast, with its massive steelworks: raw materials manufactured in these factories often contain As, that is therefore found, in variable concentrations, in fumes and solid or liquid waste, potentially contaminating the environment and posing health threats for nearby residents. The study was conducted on healthy volunteers from those areas, with different levels of As exposure and contamination.

Comparison with previous studies

Increased atherosclerosis due to exposure to As affects the carotid artery (Wang et al. 2002; Hsieh et al. 2011). Besides overt atherosclerotic plaques, increased IMT has been associated with As exposure (Chen et al. 2006; Osorio-Yanez et al. 2013).

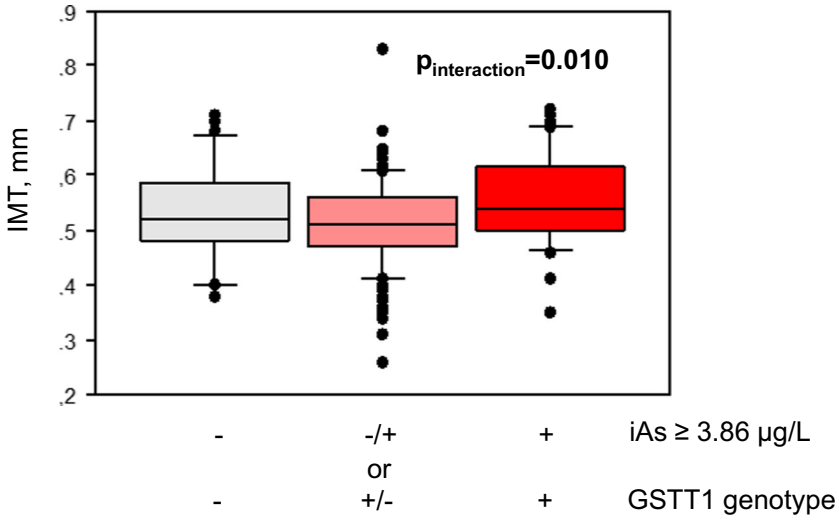

Fig. 2 Box and whiskers plot of IMT according to high urinary As level (≥3.86 μg/L) and GSTT1-positive (+)/-null (−) genotype

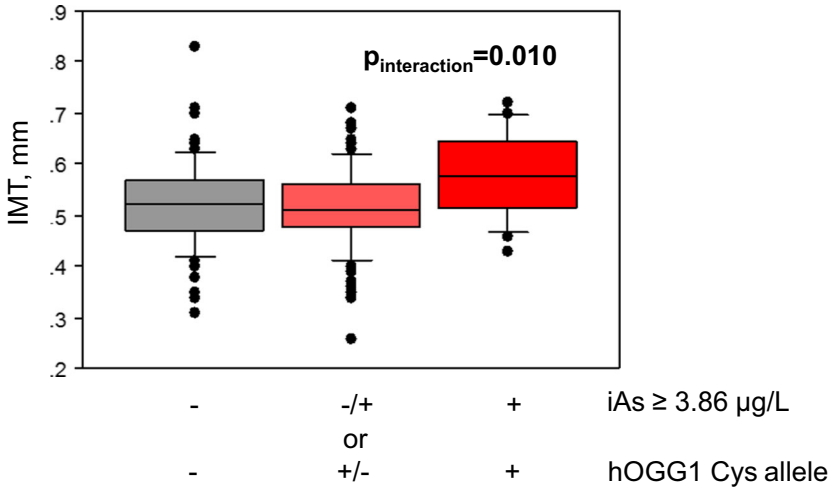

Fig. 3 Box and whiskers plot of IMT according to high urinary As level (≥3.86 μg/L) and hOGG1 Cys allele present (+)/absent (−)

Several population studies relate exposure to high levels of As to an increased incidence of ischemic heart disease and CV mortality (Stea et al. 2014). An association has been shown between exposure to As, CV risk factors such as hypertension and diabetes mellitus, and vascular damage such as carotid atherosclerosis; however, the effects of lower As levels, closer to the WHO threshold, are less explored (Moon et al. 2013; Farzan et al. 2015). Interestingly, D'Ippoliti et al. evaluated the health effects of arsenic in drinking water in the Viterbo Province with concentrations within a low-medium range, on a population with long-term exposure (40 years on average). The authors provided evidence that even at these levels, arsenic resulted associated with mortality from several chronic conditions such as myocardial infarction, peripheral arterial disease, lung cancer, respiratory diseases, and diabetes (D'Ippoliti et al. 2015).

Our data also showed significant interactions between urinary As level and GSTT1 and hOGG1 polymorphism on IMT values. These data support the evidence that individuals genetically predisposed to suboptimal or incomplete As metabolism are more susceptible to the effect of As exposure on atherosclerosis (Hsieh et al. 2011; Chiou et al. 1997; Kile et al. 2013; Wu et al. 2014). Phase II metabolic GST enzymes conjugate metabolic intermediates into more soluble forms, which are then excreted by the body. Individuals who lack the enzyme activity of GSTM1 and GTTT1 enzymes have been considered a high-risk group for carotid atherosclerosis due to their deficiency in GSTs for efficiently conjugating As with methyl groups to form hydrophilic metabolites (Wang et al. 2007).

However, in As metabolism, where the products of primary and secondary methylation (MMA^{III} and DMA^{III}) are suspected to be more reactive than their metabolic precursors MMA^{V} and DMA^{V}, individuals with high detoxifying ability may be at greater risk of adverse effects associated with chronic As exposure through drinking water (McCarty et al. 2007).

hOGG1 is a DNA repair enzyme specifically linked with the removal of 8-hydroxyguanine resulting from the action of reactive oxygen species. The Ser326Cys genetic variant is the most frequently studied SNP. Individuals with Ser/Cys or Cys/Cys hOGG1 genotypes showed slower DNA repair capacity compared to those with the Ser/Ser hOGG1 genotype.

Interestingly, the functional Ser326Cys hOGG1 genetic polymorphism has been reported to increase the risk of hypertension in individuals exposed to high level of As (Chen et al. 2012). Thus, OGG1 Cys allele carriers with attenuated DNA repair mechanisms following As exposure may be more likely to develop premature vascular aging, according to previous studies showing that DNA repair capacity is associated with accelerated vascular aging (Andreassi et al. 2015; Durik et al. 2012).

Several factors could contribute to the substantial negativity of our findings. Urinary As correlates well with recent exposure (Jomova et al. 2011), but the total lifetime exposure—the actual source of cumulative vascular damage—was not available.

IMT is an established early marker of atherosclerosis (Lorenz et al. 2007; Mancia et al. 2013) and represents a subclinical alteration of the vessel wall, detectable long before overt disease occurs. Its measurement with ultrasound is especially simple in the common carotid, where the vessel is usually straight, superficial, and without plaques. An automated edge detection software was used to obtain accurate measurements of the vessel wall, whether on-line or off-line on stored images (Faita et al. 2008) in order to reduce variability. The thickness of the intima-media layer in the present sample was small and mostly in the normal range for the healthy population (Engelen et al. 2013): the absolute magnitude of variations could be under the very resolution limit of the method. Actually, an association between IMT and As is established with higher As values and with higher average IMT (Chen et al. 2006): with lower exposures, the effect is

expected to be smaller and the contribution of other confounding factors relatively larger (Moon et al. 2012; Tsuji et al. 2014); methodological limitations—mainly due to the difficulty of assessing actual and cumulative individual exposure and the distinction between organic and inorganic As—may offset the potential detrimental effect, but more recent and accurate studies have started to shed a light on the subject (Farzan et al. 2015; Wade et al. 2015; Mendez et al. 2016; Farzan et al. 2015).

Study limitations

Several limitations have to be acknowledged to the present study. The sample adhered to the study on a voluntary basis, it is young and healthy, and it did not enter a follow-up program. Urinary arsenic was measured on a single day, and this method, although rational and widely used (Jomova et al. 2011; Osorio-Yanez et al. 2013; Ameer et al. 2015), does not accurately reflect lifetime exposure; lifetime exposure was not observed, and the potential detrimental As effect on hard clinical endpoints was not envisioned in the present protocol.

The observational and cross-sectional design of the study does not allow drawing definitive conclusions about causality or changes over time. However, the sample was recruited in different areas with different levels of As pollution in order to provide a realistic picture of such a diverse territory as Italy.

The sample is at a very low risk for CV, and an imaging biomarker such as IMT is modulated by age and also by conventional risk factors, which were not specifically assessed in this study. Regarding the increase of common carotid IMT with age, the finding—a higher slope and a higher baseline IMT in the present sample than in the reference population—is interesting and deserves attention and further research, but it cannot be substantiated by a formal statistical comparison due to unavailability of necessary data (Engelen et al. 2013) and is far from having immediate implications for public health.

Finally, established genetic variants related to As exposure were analyzed. However, a full panel of mutations related to atherosclerosis and its CV complications could not be performed.

Conclusions

In a sample of young adult volunteers from areas of Italy where the general population is potentially exposed to As, no correlation was found between urinary As and IMT of the common carotid. IMT could probably not be a marker with enough sensitivity for early CV impairment in this range of age and thickness. The analysis hints, however, at a more rapid increase of IMT with age in this population, i.e., a sign of vascular aging: how much of this that would be attributable specifically to As is not known. Our findings also suggested that GSTT1 and hOGG1 polymorphisms might play an important role in the individual risk of As-induced carotid atherosclerosis. Future research is needed to examine the possible importance of the additive effects of As exposure and genetic variants as well as to elucidate disease mechanisms and provide directions for early prevention.

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