Arsenic exposure, diabetes-related genes and diabetes prevalence in a general population from Spain☆

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A B S T R A C T

Inorganic arsenic exposure may be associated with diabetes, but the evidence at low-moderate levels is not sufficient. Polymorphisms in diabetes-related genes have been involved in diabetes risk. We evaluated the association of inorganic arsenic exposure on diabetes in the Hortega Study, a representative sample of a general population from Valladolid, Spain. Total urine arsenic was measured in 1451 adults. Urine arsenic speciation was available in 295 randomly selected participants. To account for the confounding introduced by non-toxic seafood arsenicals, we designed a multiple imputation model to predict the missing arsenobetaine levels. The prevalence of diabetes was 8.3%. The geometric mean of total arsenic was 66.0 μg/g. The adjusted odds ratios (95% confidence interval) for diabetes comparing the highest with the lowest tertile of total arsenic were 1.76 (1.01, 3.09) and 2.14 (1.47, 3.11) before and after arsenobetaine adjustment, respectively. Polymorphisms in several genes including *IL8RA, TXN, NR3C2, COX5A* and *GCLC* showed suggestive differential associations of urine total arsenic with diabetes. The findings support the role of arsenic on diabetes and the importance of controlling for seafood arsenicals in populations with high seafood intake. Suggestive arsenic-gene interactions require confirmation in larger studies.

1. Brief summary of the main result of the work

In a general population from Spain with low-moderate inorganic arsenic exposure, increased arsenic exposure, assessed as urine arsenic concentrations, was associated with higher diabetes prevalence. The reported association was stronger after adjustment for urine arsenobetaine reflecting the importance of accounting for seafood consumption in populations with high seafood intake.

Abbreviations: HbA1C, glycosylated hemoglobin; ICPMS, inductively coupled-plasma mass spectrometry; MCMC, Markov Chain Monte Carlo; SNPs, single nucleotide polymorphisms.

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Carriers of specific genotypes may have increased susceptibility to arsenic-related diabetes, although larger studies are needed to confirm these suggestive findings.

2. Introduction

Epidemiological studies support that people with higher inorganic arsenic exposure levels are more likely to have diabetes (Navas-Acien et al., 2006) but the evidence at low to moderate exposure levels is not sufficient. Moreover, most studies have been conducted in Asia and the Americas, with very few studies on arsenic and diabetes being conducted in Europe. Epidemiological studies in diverse populations are needed given the elevated burden of diabetes, a world-wide rapidly growing disease (World Health Organization, 2016).

The sum of urine concentrations of inorganic arsenic (arsenite [AsIII] plus arsenate [AsV]) and its methylated species (monomethylarsonate [MMA] and dimethylarsinate [DMA]) is an established biomarker of inorganic arsenic exposure in populations with low seafood intake (Navas-Acien et al., 2011). It is well known that inorganic arsenic (iAs) is highly toxic to humans (World Health Organization, 2001) and is associated with a wide range of adverse health effects, including cancer (Bates et al., 1992), cardiovascular disease (Moon et al., 2012) and others (D'Ippoliti et al., 2015). People are exposed to inorganic arsenic mainly through drinking contaminated water (National Research Council, 1999) and through food intake (Wei et al., 2014). Organic arsenic species, such as arsenobetaine (Asb), arsenolipids and arsenosugars, are mostly found in saltwater finfish and shellfish and are considered not harmful to human health (Mozaffarian and Rimm, 2006). Among the seafood arsenicals, arsenobetaine is the most common compound, which is rapidly cleared from the bloodstream and excreted unchanged in the urine (Molin et al., 2014), contributing to the total urinary arsenic concentrations. Arsenosugars and arsenolipids are metabolized in the body resulting in multiple metabolites in the urine, including DMA, and contributing to the total amount of inorganic arsenic species. As a result, in the presence of seafood intake, it is necessary to account for the potential residual confounding of organic arsenic in the interpretation of urinary arsenic concentrations as a biomarker of inorganic arsenic exposure (Navas-Acien et al., 2011).

The objective of this study was to evaluate the association of inorganic arsenic exposure, assessed as total urinary arsenic concentrations, with the prevalence of type 2 diabetes in a population from Spain with relatively high seafood consumption. This research was complicated by the presence of missing completely at random urine arsenobetaine concentrations for the ~80% of the study sample. We thus developed and implemented a multiple imputation model based in Markov Chain Monte Carlo (MCMC) methods using Gibbs sampling to account for the potential residual confounding by organic arsenicals in the whole study population. Given the well-established role of genetic variation on diabetes traits (Mahajan et al., 2014) and the paucity of gene-environmental interaction studies, we also evaluated the interaction of arsenic and diabetes-related candidate polymorphisms as a potential determinant of diabetes.

3. Methods

3.1. Study population

The present study was conducted among adult participants of the Hortega Study, who were beneficiaries of the public health system assigned to the Rio Hortega University Hospital's catchment area (Valladolid, northwestern Spain). The Hortega Study uses a complex sampling design to obtain a representative sample of the general population. The sampling design and methodology have been previously described (Mena Martin et al., 2003). In 2001–2003, baseline information on socio-demographic, behavioral, dietary and other health-related factors were collected from in-person interviews, examinations, and review of health records (see e-Appendix 1). After signing an informed consent form, urine and blood samples were collected and stored at −80 °C. A total of 1502 individuals had sufficient urine and plasma samples available for metal determinations. After excluding 21 participants with missing total arsenic concentrations, 2 missing urine creatinine, 10 missing seafood intake, and 18 missing other relevant covariates, 1451 participants were included in the present study.

3.2. Arsenic measurements and arsenobetaine imputation

Urine arsenic determinations were conducted by the Laboratory of Analytical and Bioanalytical Chemistry of Huelva University (Spain) in 2013. Total urine arsenic and arsenic species concentrations were measured by inductively coupled-plasma mass spectrometry (ICPMS) on an Agilent 7500CEx ICPMS (Agilent Technologies, Santa Clara, California) and by anion exchange liquid chromatography coupled to ICPMS with octapole reaction cell, respectively, following a standardized protocol (see e-Appendix 2). Urine arsenic speciation, including AsIII, AsV, MMA, DMA, and Asb concentrations, occurred only in a random subsample of 295 individuals, leaving 1156 (79.7%) of the 1451 participants with missing completely at random urine arsenic species concentrations. Asb and DMA levels below the limit of detection (12 and 1 undetectable values, respectively) were imputed by the limit of detection divided by the square root of 2 (Hornung RW and Reed LD, 1990). Total plasma arsenic levels were measured in the whole study population in 2010 by atomic absorption spectrometry with graphite furnace at Cerba International Laboratories Ltd. 42.6% of total plasma arsenic concentrations were under the limit of detection. See limits of detection in e-Appendix 2.

We used the arsenic speciation information available in the random subsample to generate 5 complete datasets (for the 1451 participants) in which the Asb values missing completely at random where imputed as the 30^{th}, 40^{th}, 50^{th}, 60^{th} and 70^{th} percentiles of each subject-specific posterior distribution obtained from a MCMC by Gibbs sampling nested linear model (Tellez-Plaza et al., 2010), implemented with WinBUGS software (Lunn et al., 2000) (see e-Appendix 3). Total plasma arsenic and DMA where strongly correlated with Asb (spearman correlation coefficients = 0.65 and 0.54, respectively). Thus, we incorporated the correlation of total plasma arsenic, DMA and Asb conditionally in our imputation model. We did not considered AsIII, AsV and MMA as potential Asb predictors. As a result, the MCMC arsenobetaine imputation model consisted of a tri-variate model with nested equations for Asb, DMA and total plasma arsenic, each based on strong predictors selected by a backward stepwise process starting from total urine concentrations and a list of socio-demographic and established arsenic exposure determinants (age, gender, smoking status, cotinine, body mass index, seafood, rice and chicken consumption) (e-Appendix 3, e-Table 1). For Asb and DMA, the predictive equations were standard linear models, whereas for total plasma arsenic the predictive equation was a tobit linear model for truncated data. The WinBUGS code of the imputation model is provided in e-Appendix 3. We conducted a *post-hoc* validation process to measure the predictive error of the MCMC imputation model using data from the Aragon Workers Health Study, an independent dataset with data on total urine arsenic, urine arsenic species and total plasma arsenic (e-Appendix 4 and e-Table 2).

3.3. Diabetes determination

All participants provided blood samples after an average time of 3 h from fasting (range 0–17). Plasma glucose was measured by an automated analyzer based on the glucose oxidase method. Participants with non-fasting glucose ≥140 mg/dL in the first determination, underwent an additional measure of glucose in fasting conditions. Glycosylated hemoglobin (HbA1c) was measured from capillary blood samples using a DCA 2000 HbA1c analyzer (Mena Martin et al., 2003). Prevalent type 2 diabetes was defined as fasting glucose levels ≥126 mg/dL, or HbA1c > 6.5%, or by physician diagnosis or glucose-lowering medication use.

3.4. Single nucleotide polymorphisms selection and genotyping

We selected 155 candidate genes, including 597 single nucleotide polymorphisms (SNPs), which were related with diabetes metabolic pathways. Detailed methods on DNA isolation and SNP genotyping have been previously published (Galan-Chilet et al., 2017). The mean genotyping coverage across all genotyped SNPs was 95%. Among the 597 selected SNPs, we excluded 42 because the coverage in the study sample was less than 90%, 59 with less than 3 genotypes, 25 with minor allele frequency <5%, 41 that did not meet the Hardy–Weinberg equilibrium ($P < .01$), and 76 with minor genotype frequency <20 individuals. 354 SNPs were finally included in gene-environment interaction analyses. The complete list of the selected genes and SNPs is provided in e-Table 3.

3.5. Statistical methods

Due to the different selection probabilities of the Hortega Study participants, all analyses were weighted to the underlying adult population aged 20 or older. Total urine arsenic and Asb concentrations were divided by urine creatinine and log-transformed for statistical analyses. We estimated the odds ratios and 95% confidence intervals (95% CI) for the association between total urine arsenic and diabetes prevalence using progressively adjusted logistic regression models. Model 1 was adjusted for age, gender and education. Model 2 was further adjusted for urine cotinine levels, smoking status, alcohol consumption, fish consumption and residence place. Model 3 was additionally adjusted for urine Asb levels. Total urine arsenic was modeled as tertiles and as restricted quadratic splines (see e-Appendix 5). We also explored whether the association between diabetes and arsenic was modified by gender, smoking status, abnormal albuminuria and reduced glomerular filtration rate status by including the interaction term of continuous total urine arsenic and the corresponding group variable in fully-adjusted models. Gene-environment interaction analyses were conducted by including each SNP and its interaction term with total urine arsenic levels. For each SNP, we estimated three separate models assuming dominant, recessive and additive inheritance. If only one model showed statistically significant SNP-arsenic interactions, we reported the associations in strata defined by the corresponding genotypes for this specific model. In cases where more than one model met statistically significant SNP-arsenic interactions, we reported the best fitting model selected by comparing to a general model that included separate dummy variables for the heterozygote and minor allele homozygote (reference major allele homozygote). The effective SNP number obtained with Plink software (Purcell et al., 2007) was 242, therefore we used a Bonferroni-corrected significance level of 0.0002 (0.05 divided by 242).

After the imputation of Asb and DMA missing completely at random and plasma arsenic levels below the detection limit, we obtained 5 databases with complete data for total urine arsenic and Asb concentrations. All statistical analyses above described were performed in each of the 5 data sets, and the results were combined using the method proposed by Rubin for multiple imputation procedures (Rubin, 1978). In sensitivity analysis, we also evaluated the association of urine total arsenic and the sum of inorganic and methylated arsenic species in urine with diabetes within the subset of 295 individuals with arsenic speciation data (e-Table 4). See e-Appendix 5 for additional sensitivity analyses. All statistical tests were two-sided.

4. Results

4.1. Participant characteristics

Among the 1451 participants, 120 (8.3%) had prevalent type 2 diabetes (Table 1). Participants with type 2 diabetes were more likely to be men, older, former smoker, with lower education and to have lower fish intake. The overall geometric mean of total plasma arsenic and total urine arsenic in the complete study population were 2.6 μg/L and 66.0 μg/g, respectively. The overall geometric mean of the sum of inorganic arsenic species, i.e, AsIII + AsV + MMA + DMA (ΣiAs), and arsenobetaine in the randomly selected subsample of 295 participants was 11.1 μg/g and 47.3 μg/g, respectively. Participants with diabetes were older and showed higher values of all arsenic biomarkers (3.8 μg/L of total plasma arsenic, 106 μg/g of total urine arsenic, 14.9 μg/g of ΣiAs and 66.5 μg/g of Asb).

4.2. Total urine arsenic and diabetes prevalence

We found a positive significant association of total urine arsenic with diabetes prevalence before and after adjustment for arsenobetaine concentrations (Table 2). The fully adjusted odds ratio (95% CI) for diabetes comparing the second and the third tertiles with the first one of total urine arsenic were 2.03 (1.63, 2.53) and 2.14 (1.47, 3.11), respectively (Table 2, model 3). In model 2, which was not adjusted for Asb, this relation was weaker and not strictly increasing (odds ratio (95% CI) comparing the second and third tertiles to the lowest were 1.85 (1.04, 3.30) and 1.76 (1.01, 3.09), respectively). The analyses repeated in the random subsample with originally measured arsenic species showed consistent findings (e-Table 4). Flexible models based on splines confirmed that multivariable adjusted odds ratio were higher after adjusting for Asb (model 2 versus model 3) (Fig. 1). No significant differences for the odds ratio of diabetes were found for gender, ever-smoking status and abnormal albuminuria subgroups (Table 3). For reduced glomerular filtration rate status, however, the association between arsenic and diabetes was stronger among participants without kidney disease (P of interaction = 0.02).

4.3. Gene-environment interaction

In gene-environment interaction analyses, while no polymorphism showed significant interactions with total urine arsenic after correcting for multiple comparisons, several genotypes showed marginally significant differential associations with diabetes (Fig. 2 and Table 4). The top 5 genes showing the highest statistical interactions were *IL8RA* (rs1008562 [$P = .004$]), *TXN* (rs4135168 [$P = .004$]), *NR3C2* (rs13117325 [$P = .007$]), *COX5A* (rs1133322 [$P = .01$]) and *GCLC* (rs11415624 [$P = .01$]).

5. Discussion

We found a positive association between increasing levels of total urine arsenic and the prevalence of type 2 diabetes in a

Table 1
Participant characteristics by overall and type 2 diabetes status.

Characteristics	No T2D	T2D	Overall
	(N = 1331)	(N = 120)	(N = 1451)
Age (years); mean (SE)	48.5 (0.2)	67.1 (1.4)	49.7 (0.2)
Gender (male); % (SE)	48.2 (0.3)	60.6 (4.6)	49.0 (0.0)
Education (<secondary education); % (SE)	20.9 (1.0)	43.8 (4.8)	22.4 (1.0)
Body mass index (kg/m^2); mean (SE)[a]	26.0 (0.1)	28.7 (0.5)	26.2 (0.1)
Urine cotinine ng/mL; % (SE)			
< 34	72.7 (1.3)	85.1 (3.8)	73.5 (1.2)
34-500	5.6 (0.7)	4.9 (2.2)	5.6 (0.6)
> 500	21.7 (1.2)	10.1 (3.4)	20.9 (1.1)
Smoking status; % (SE)			
Never	44.5 (1.3)	46.1 (4.8)	44.6 (1.3)
Former	27.9 (1.2)	39.8 (4.8)	28.6 (1.2)
Current	27.7 (1.3)	14.1 (3.8)	26.8 (1.2)
Alcohol intake (mg/day); mean (SE)	11.2 (0.6)	11.2 (2.9)	11.2 (0.6)
Fish intake (g/month); % (SE)			
0	12.4 (0.9)	24.0 (4.2)	13.1 (0.9)
0–2000	46.0 (1.4)	32.4 (4.6)	45.2 (1.3)
> 2000	41.6 (1.4)	43.7 (4.9)	41.7 (1.3)
Residence place (urban); % (SE)	76.6 (1.2)	75.8 (4.3)	76.6 (1.1)
Total plasma arsenic (μg/L); GM (95% CI)	2.5 (2.4, 2.7)	3.8 (3.0, 4.7)	2.6 (2.4, 2.7)
Total urine arsenic (μg/L); GM (95% CI)	59.5 (55.3, 64.0)	106.4 (82.6, 137)	61.7 (57.5, 66.2)
Urine creatinine (g/L); GM (95% CI)	0.93 (0.90, 0.96)	1.01 (0.91, 1.12)	0.94 (0.91, 0.96)
Total urine arsenic (μg/g); GM (95% CI)	64.0 (59.0, 69.3)	106 (82.3, 136)	66.0 (61.1, 71.2)
ΣiAs (μg/g); GM (95% CI) [b,c]	10.9 (9.2, 12.9)	14.9 (11.0, 20.2)	11.1 (9.5, 13.0)
Arsenobetaine (μg/g); GM (95% CI) [c]	46.1 (37.5, 56.7)	66.5 (37.9, 116.6)	47.3 (38.8, 57.6)
% iAs; median (IQR) [c]	2.7 (1.1, 5.8)	1.9 (1.4, 5.0)	2.6 (1.2, 5.6)
% MMA; median (IQR) [c]	11.0 (2.7, 29.7)	6.3 (2.5, 18.5)	10.4 (2.7, 29)
% DMA; median (IQR) [c]	83.7 (61.1, 94.3)	90.8 (79.5, 94.7)	84.6 (62.5, 94.3)

AsIII, AsV, MMA, DMA and Asb not detectable values were imputed as a LOD divided by the square root of 2.; Abbreviations: T2D, type 2 diabetes; SE, standard error; GM, geometric mean; CI, confidence interval; IQR, interquartile range; AsIII, arsenite; AsV, arsenate; iAs, inorganic arsenic, MMA monomethylarsonate; DMA, dimethylarsinate; Asb, arsenobetaine.

[a] 6 and 50 participants with and without T2D missed body mass index information.

[b] ΣiAs = AsIII +AsV + MMA + DMA; iAs = AsIII + AsV.

[c] Calculated in the 295 participants subsample with available arsenic speciation measurements (27 and 268 participants with and without T2D).

Table 2
Odds ratio (95% confidence interval) for type 2 diabetes by total urine arsenic concentrations (N = 1451).

Total Urine Arsenic, μg/g	Cases/Non cases	Model 1[a]		Model 2[b]		Model 3[c]	
		OR	95% CI	OR	95% CI	OR	95% CI
Tertile 1 (0–33.77)	25/437	1 Ref.		1 Ref.		1 Ref.	
Tertile 2 (33.77–123.90)	41/432	1.85	1.04, 3.28	1.85	1.04, 3.30	2.03	1.63, 2.53
Tertile 3 (>123.90)	54/462	1.75	1.01, 3.03	1.76	1.01, 3.09	2.14	1.47, 3.11
p80th vs. p20th	120/1331	1.30	0.94, 1.80	1.31	0.94, 1.81	1.76	1.54, 2.02

AbbreviationsOR, odds ratio; CI, confidence interval.
p80th and p20th are 229.93 and 19.25 μg/g of creatinine, respectively.

[a] Model 1: Adjusted for age (years), gender (male, female) and education (<secondary education, >= secondary education).

[b] Model 2: Model 1 further adjusted for urine cotinine levels (<34, 34–500 and > 500 ng/mL), smoking status (non-smoker, former and current), alcohol consumption (mg/day), fish consumption (g/month), and residence place (rural, urban).

[c] Model 3: Model 2 further adjusted for log-transformed urine arsenobetaine levels (μg/g).

representative sample of Spanish adults characterized by exposure to low arsenic levels. Our results support the relevance of adjusting for arsenobetaine levels in populations with high seafood consumption in order to estimate the association with arsenic exposure not derived from seafood. We also observed suggestive statistical interactions of urine arsenic levels with genetic variation in several genes involved in diabetes metabolic pathways, especially genes involved in regulation of oxidative stress response or inflammation.

The arsenobetaine levels in our study population (median: 160.3 μg/L, interquartile range: 15.8–124.5 μg/L) were much higher than those observed in a study of the 2003–2004 National Health and Nutrition Examination Survey (NHANES) (median: 0.9 μg/L, interquartile range: 0.3–3.5 μg/L) (Navas-Acien et al., 2008), reflecting a higher seafood intake. In populations with low seafood consumption, subtracting arsenobetaine concentration from total urine arsenic levels may provide a valid estimate of inorganic arsenic, since it removes the most common part of organic arsenic in seafood (Steinmaus et al., 2009). In populations with high seafood consumption, however, this approach may not be sufficient to remove the contribution of other seafood organic arsenic compounds, such as arsenosugars and arsenolipids and their metabolites, including DMA (Navas-Acien et al., 2011). Arsenobetaine is specific to seafood, it is excreted in the urine untransformed, and it is correlated with other seafood arsenicals. Using arsenobetaine as a covariate in the statistical regression models can thus indirectly control for other seafood arsenic species and their metabolites (Navas-Acien et al., 2009).

In 2003, the maximum admissible arsenic levels in drinking water in Spain decreased from 50 μg/L to 10 μg/L (Ministerio de la

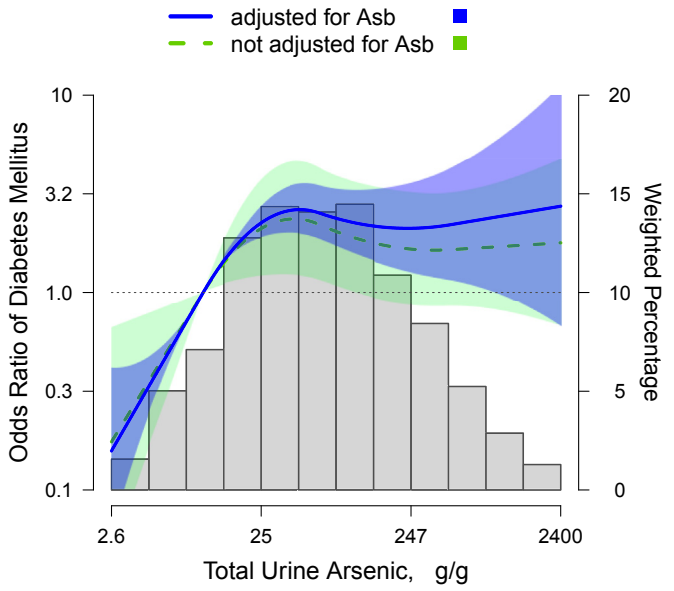

Fig. 1. Odds ratio of type 2 diabetes by total urine arsenic concentrations, before and after adjustment for arsenobetaine (Asb). Curves represent the odds ratios for type 2 diabetes based on restricted quadratic splines with knots at the 10th, 50th, and 90th percentiles (10.4, 61.1 and 450.1 μg/g, respectively) of the total urine arsenic distribution. The reference was set at the 10th percentile of the total urine arsenic distribution. Dotted line and light gray area represent the odds ratio and the 95% confidence interval before adjustment for arsenobetaine levels, i.e., adjusted for age, gender, education (<secondary education, ≥secondary education), urine cotinine levels (<34, 34–500 and > 500 ng/mL), smoking status (non-smoker, former, current), alcohol intake, fish intake, and residence place (rural, urban). Solid line and dark gray area represent the odds ratio and the 95% confidence interval after further adjustment for log-transformed urine arsenobetaine (μg/g). Bars represent the weighted distribution of creatinine corrected total urine arsenic concentrations (μg/g). Tails were truncated by excluding 1% of the study sample with total urine arsenic concentrations below 2.6 μg/g and 1% of the study sample with total urine arsenic concentrations above 2400 μg/g.

Table 3
Odds ratio (95% confidence interval) for type 2 diabetes comparing the 80th and the 20th percentiles of total urine arsenic by participant subgroups (N = 1451).

	N	Cases/Non cases	OR	95% CI	Interaction *P*
Overall	1451	120/1331	1.76	1.54, 2.02	
Gender					
Female	722	46/676	1.68	1.45, 1.94	
Male	729	74/655	1.85	1.57, 2.19	0.77
Smoking					
Never	681	59/622	2.08	1.78, 2.42	
Ever	770	61/709	1.52	1.30, 1.78	0.35
Abnormal Albuminuria					
No	1421	108/1313	1.92	1.67, 2.21	
Yes	26	14/12	2.79	1.67, 4.66	0.61
Reduced GFR					
No	1331	94/1237	2.19	1.89, 2.54	
Yes	120	26/94	0.99	0.85, 1.15	0.02

Abbreviations: OR, odds ratio; CI, confidence interval; GFR, glomerular filtration rate. p80th and p20th are 229.93 and 19.25 μg/g of creatinine, respectively. Models were adjusted for further adjusted for age (years), gender (male, female), educational level (<secondary education, >= secondary education), urine cotinine (<34, 34–500 and > 500 ng/mL), smoking status (non-smoker, former and current), alcohol consumption (mg/day), fish consumption (g/month), residence place (rural, urban), and log-transformed urine arsenobetaine levels (μg/g).

Presidencia, 2003) as recommended by the World Health Organization. Our study participants were recruited between 1997 and 2003 in Valladolid, in central Spain. Most participants were most likely exposed to low levels of inorganic arsenic in drinking water. It is possible, however, that some participants coming from rural areas could have been exposed to moderate-high arsenic levels from the groundwater. A study evaluating groundwater quality in rural areas of Valladolid found concentrations of arsenic reaching levels as high as 260 μg/L in 2001 (mean = 93 μg/L), 187 μg/L in 2003 (mean = 48 μg/L) and 51 μg/L in 2007 (mean = 15 μg/L) (Mayorga et al., 2014). In our study population, the sum of inorganic and methylated arsenic concentrations was similar in participants from rural (geometric mean = 8.8 μg/g) and urban areas (geometric mean = 11.9 μg/g), even after adjustment for Asb and seafood intake (data not shown).

Several epidemiologic studies have found positive associations between arsenic exposure levels and diabetes in adults, both cross-sectionally (Navas-Acien et al., 2008) and prospectively (Brauner et al., 2014; James et al., 2013; Kim et al., 2013). In the US, increasing levels of urine arsenic were associated with increasing odds of diabetes after adjustment for urine arsenobetaine (Navas-Acien et al., 2008). Long-term exposure to low arsenic levels, assessed as arsenic concentrations in drinking water, has been prospectively associated with diabetes in central Italy (D'Ippoliti et al., 2015) and Denmark (Brauner et al., 2014). Studies using biomarkers of arsenic exposure also found positive relationship between arsenic and incident diabetes in Colorado (James et al., 2013) and American Indians from South-West of the United States (Kim et al., 2013), but not in American Indians 45–74 years of age participating in the Strong Heart Study (Kuo et al., 2015).

Experimental evidence supports the role of arsenic as a diabetes risk factor. In rodents, exposure to arsenic through contaminated water has shown to induce insulin resistance (Palacios et al., 2012) and beta-cell dysfunction (Liu et al., 2014). The liver is the major target organ of the regulation of arsenic exposure and metabolism levels, and several experimental studies have demonstrated that chronic arsenic exposure induces severe toxic effects in the liver (Santra et al., 1999; Mazumder, 2005). The liver also plays a major role in the regulation of glucose metabolism (Cotrozzi et al., 1997). *In vivo* evidence suggests that arsenic-induced diabetes may be mediated by increased oxidative stress in hepatic and pancreatic tissue (Patel and Kalia, 2013). Interestingly, in our gene-environment interaction analysis, we observed differential associations of arsenic with polymorphisms in genes involved in redox-related pathways, including SNPs in *TXN* and *GCLC*, genes that are highly expressed in the pancreas and liver (Diaz et al., 2002; Tran et al., 2004; Yamamoto et al., 2008; Okuyama et al., 2008), although the evidence was only suggestive. Some studies, however, support a potential biological link between proteins encoded by these genes and arsenic.

A positive correlation was observed between serum thioredoxin1 (*TXN1*) and total water arsenic intake or urinary arsenic species in humans (Li et al., 2012). Arsenic inhibits thioredoxin reductase, and this may contribute to *TXN* oxidation (Lin et al., 2001). Another mechanistic study showed that arsenic exposure can disrupt the *GCLC* expression, since low arsenic exposure increased the expression of *GCLC* in liver tissues of rats while high arsenic exposure levels reduced its expression (Ren et al., 2015). In addition, the interaction of arsenic with the polymorphism rs1008563 in *IL8RA* (also known as *CXCR2*), a chemokine receptor for interleukin-8 (*IL8*), showed the highest p-value. Several lines of evidence support that *IL8RA* inhibitors play a key role in the etiopathogenesis of type 1 diabetes (Diana et al., 2013; Valle et al., 2013). Interestingly, an experimental study found that urothelial cells chronically exposed to trivalent monomethylarsonate showed an over-expression of *IL8* (Escudero-Lourdes et al., 2012).

Despite the limited power to detect interactions at the Bonferroni-corrected level, we found marginally significant associations for some polymorphisms, which, given the biological relevance on diabetes-related pathways, should be evaluated in

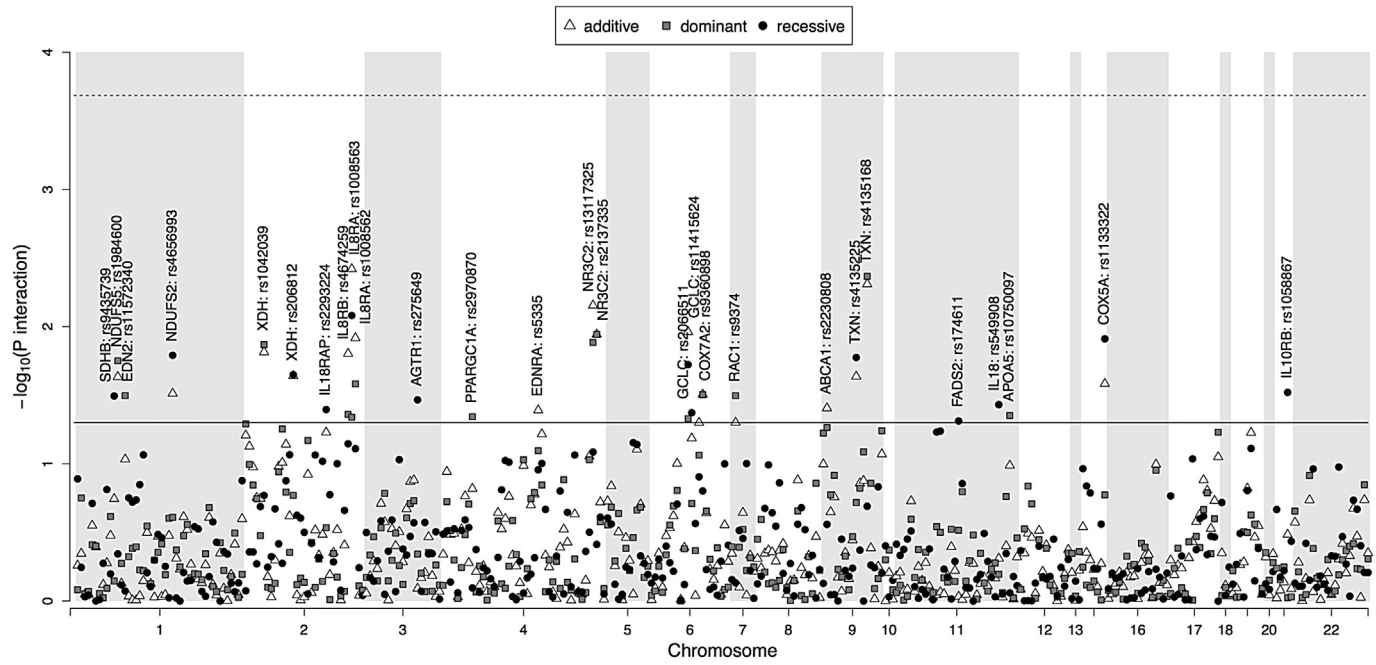

Fig. 2. Candidate genes-arsenic interaction $-\log_{10}$ *P*-values. P-values for 354 SNPs derived from linear regression models (dominant, recessive and additive model) adjusted for age, sex, education, urine cotinine levels, smoking status, alcohol consumption, fish consumption, residence place and log-transformed arsenobetaine, are presented on the left Y axis on the logarithmic scale according to the position of the SNPs on chromosome (X axis). The horizontal solid line corresponds to a nominal p-value of significance equal to 0.05. Horizontal dashed line corresponds the effective SNP number corrected p-value equal to 0.0002.

Table 4
Odds ratio (95% CI) of type 2 diabetes comparing the 80th *vs* the 20th percentiles of total urine arsenic distribution by genotypes among the 5 genes with top interaction p-values.

Chr.	Gene	SNP	Model	Cases/Non cases	Genotype	OR	95% CI	*P* Interaction
2	**IL8RA**	rs1008563	ADD	40/329	T/T	2.26	2.18, 2.34	0.004
				46/608	T/C	3.38	3.29, 3.46	
				27/313	C/C	5.04	4.69, 5.42	
		rs1008562	ADD	49/451	C/C	2.48	2.40, 2.56	0.01
				44/602	C/G	3.48	3.39, 3.58	
				20/207	G/G	4.89	4.48, 5.33	
9	**TXN**	rs4135168	DOM	71/732	A/A	4.19	4.01, 4.38	0.004
				47/566	A/G + G/G	2.48	2.38, 2.58	
4	**NR3C2**	rs13117325	ADD	48/572	G/G	3.87	3.73, 4.02	0.007
				46/554	G/A	2.82	2.76, 2.88	
				18/138	A/A	2.06	1.93, 2.19	
		rs2137335	DOM	82/779	T/T	2.70	2.63, 2.76	0.01
				33/492	T/C + C/C	4.65	4.30, 5.03	
6	**GCLC**	rs11415624	ADD	58/585	D/D	3.80	3.67, 3.93	0.01
				54/574	D/A	2.73	2.67, 2.79	
				8/164	A/A	1.96	1.84, 2.09	
15	**COX5A**	rs1133322	REC	96/978	T/C + T/T	3.44	3.36, 3.53	0.01
				21/323	C/C	2.03	1.90, 2.17	

Abbreviations: OR, odds ratio; CI, confidence interval; ADD, DOM, and REC, additive, dominant and recessive model, respectively.
The Bonferroni-corrected significance level was 0.0002.
Odds ratio for diabetes comparing the 80th and 20th percentiles of urine arsenic distributions (229.93 and 19.24 μg/g respectively) and associated test for interaction were obtained from linear regression models with log-transformed arsenic as a continuous variable. Models were adjusted for age (years), sex (male, female), education (<secondary education, >=secondary education), urine cotinine levels (<34, 34–500 and > 500 ng/mL), smoking status (non-smoker, former and current), alcohol consumption (mg/day), fish consumption (g/month), residence place (rural, urban) and log-transformed arsenobetaine (μg/g).

larger studies. There are limited studies evaluating the interaction of genetic variation and arsenic on diabetes. A prospective study in Bangladesh (N = 957) reported a significant interaction of SNPs in *NOTCH2* with arsenic exposure (assessed in drinking water) on the association with type 2 diabetes (Pan et al., 2013). Unfortunately, we did not genotype candidate SNPs in *NOTCH2*. Additional and larger prospective studies, especially with an extensive panel of candidate genes, are needed.

One of the limitations of our study is the cross-sectional design, which cannot determine the direction of the associations. Elevated arsenic exposure levels could increase diabetes risk by inducing liver damage, as well as liver dysfunction related to diabetes might influence arsenic metabolism and result in higher arsenic excretion in the urine (Ahmadieh and Azar, 2014). Another limitation is the use of one single urine sample given the within-subject variability in urinary arsenic excretion. Our study has also some strengths, including the complex sampling design; the adjustment for relevant diabetes risk factors, potential confounders and markers of

seafood intake; the use of a multiple imputation model to adjust total urine arsenic for arsenobetaine, which enabled the use of urinary arsenic as a proxy for inorganic arsenic exposure; and the availability of SNPs in 155 candidate genes for gene-environmental evaluation.

In conclusion, arsenic exposure was associated with increased prevalence of type 2 diabetes in a general population from Spain. Our findings support the hypothesis that arsenic plays a biological role as a diabetogenic risk factor. Our analytical strategy also shows the importance of controlling for seafood arsenicals in populations with high seafood consumption. While the evaluated gene-environment interactions were only close to statistical significance after multiple comparisons correction, and should be only considered exploratory, our results provide the basis for gene-environment interaction studies in larger epidemiologic studies.

Conflicts of interest

All authors declared that they do not have any conflict of interest.

Data and computing code

Code for MCMC imputation is provided in the Supplemental Material. The computing code for other statistical models is available upon request to the corresponding author. The individual-level data are not available as the steering committee and the participants did not approve unrestricted data sharing at the time of ethical approval of the study and data sharing was not included in the consent form.

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Appendix. Supplementary Material

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.01.008.

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