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Assessment of YAP gene polymorphisms and arsenic interaction in Mexican women with breast cancer

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Abstract

The identification of gene-environment interactions related to breast cancer reveals the biological and molecular mechanisms underlying the disease and allows distinction of women at high risk from women at lower risk, which could decrease the morbi-mortality of this neoplasm. The current study evaluated the association between polymorphisms rs1820453 and rs11225161 of the YAP

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gene in women with breast cancer exposed to arsenic (As) through drinking water. The frequency of YAP rs1820453 and rs11225161 polymorphisms and As urinary levels were assessed in a total of 182 women. The results demonstrated a positive and significant association between breast cancer and smoking, type of drinking water, and levels of As^{III}, As^{V} and inorganic arsenic (iAs) but not the YAP gene polymorphisms evaluated. In conclusion, our data showed that the source of drinking water and As^{V} and iAs urinary levels increase the risk for breast cancer, but no interactions between YAP gene polymorphisms and arsenic urinary levels were found.

Short Abstract

No associations were found between BC with YAP gene polymorphisms. An increased risk for BC with type of drinking water, As^{V} and iAs was registered and no interactions between YAP polymorphisms with arsenic urinary levels were documented.

Keywords

Breast cancer; arsenic; Yes Associated Protein; YAP; YAP rs1820453 polymorphism; YAP rs11225161 polymorphism

Introduction

Breast cancer (BC) remains a major public health problem and is the most frequently diagnosed cancer and the leading global cause of cancer death in women, accounting for 23% of cancer diagnoses (1.38 million women) and 14% of cancer deaths (458,000 women) each year (Jemal et al., 2011). Breast cancer is the leading cause of cancer mortality among women of reproductive age, and an increase in its incidence has been documented in Mexico (Valencia-Mendoza et al., 2009) and in most developed and non-developed countries.

The process of carcinogenesis is complex, and it is believed that multiple mechanisms contribute to the development of cancer with disruption of the equilibrium between cellular proliferation and apoptosis (Yu and Guan, 2013). Due to these multiple mechanisms, the identification of gene-environment interactions related to breast cancer could supply insight into the biological mechanisms underlying the disease (Barrdahl et al., 2017).

Among a large quantity of genes implicated in the cancer process, the Hippo pathway has generated considerable interest in recent years because of its involvement in several key hallmarks of cancer progression and metastasis (Cao and Huang, 2017). Components of the Hippo pathway were identified in Drosophila by genetic screens in 1995 and linked for the first time to human cancer in 2002 (Ikeda and Sadoshima, 2016; Tapon et al., 2002). Among the main components of Hippo pathway is the mammalian transcriptional coactivator Yes-associated protein (YAP). This protein is considered as a nuclear effector of the Hippo pathway (Dong et al., 2007; Pan, 2007), and it promotes expression of genes involved in cell proliferation and apoptosis suppression (Zhao et al., 2009; Zhu et al., 2015), but its dysregulation might contribute to a malignant cellular phenotype (Dong et al., 2007). YAP dysregulation has been observed in many human cancers (liver, stomach, ovary, prostate, colon, esophagus, breast) and in glioblastomas (Su et al., 2012; Wang et al., 2010), but little

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is known of its role in breast tissue. With respect to this last point, a few contrasting reports exist related to its function because it has been proposed to act as both a tumor suppressor and an oncogene (Kim et al., 2015). Recently, via immunohistochemistry, our group assessed YAP expression at the nucleus and in the cytoplasm in breast biopsies from women with and without BC. We found a significantly lower percentage of YAP cytoplasm expression in BC samples and observed that YAP high-intensity staining in the cytoplasm was negatively associated with breast cancer (Michel-Ramirez et al., 2017).

Genetic variants that contribute to the susceptibility of sporadic BC are still limited, unclear or unknown. A few reports associating YAP gene polymorphisms with BC or other reproductive pathologies have been reported, and among them, Chen et al. (2013), identified a significant association between the YAP rs1820453 polymorphism with BC in a Chinese population, and Li et al. (2012) found an association between the YAP1 rs11225161 polymorphism with polycystic ovary syndrome.

In addition to the YAP functions mentioned previously, YAP is crucial in the response to oxidative stress induced by cellular processes and by different xenobiotics as well as the development of resistance to cytotoxic agents and heavy metals (Toone and Jones, 1999). More than 200 million people in 70 countries are exposed to arsenic (a naturally occurring metalloid and Class-A human carcinogen) through drinking water (Minatel et al., 2018). It has been reported that As induces the Hippo signaling pathway and contributes to aberrant activation, which in turn could contribute to the pathogenesis of epithelial neoplasm (Li et al., 2013). Arsenic exposure has been recognized as a contributor to the etiology of BC. High urinary As levels have been detected in BC patients compared with those found in controls (Benderli Cihan et al., 2011; Joo et al., 2009). In a study of Polish women, Muszy ska et al. (2012) reported an increased risk for BC in those with higher serum arsenic levels and BRCA1 mutation carriers.

Recently, our group studied YAP expression in breast cancer tissue from women chronically exposed to As through drinking water. We suggested that YAP might act as a tumor suppressor protein and that As is able to reduce YAP translocation from the cytoplasm to the nucleus, which can induce a favorable environment for inhibition of apoptosis and promotion of cellular proliferation by increasing the genetic instability of cells that might contribute to the pathogenesis of cancer (Michel-Ramirez et al., 2017).

The current study was designed with the aim of increasing knowledge of gene-environment interactions because of a lack of previous studies associating the YAP gene rs1820453 and rs11225161 polymorphisms with arsenic exposure in women with breast cancer.

Materials and Methods

Study population

A cross-sectional study was implemented, and participants were recruited from the Department of Gynecologic-Oncology, Mexican Institute of Social Security, Torreon, Coahuila, Mexico. The volunteers were residents of the Comarca Lagunera region, where high arsenic tap water levels have been detected (Recio-Vega et al., 2015). The Comarca

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Lagunera is located in the north-central region of Mexico and known as an area where increased arsenic toxicity has been reported (Sampayo-Reyes et al., 2010; Recio-Vega et al., 2015).

Eligible patients included women over 18 years old who had at least 10 years of residence in this geographical area. We excluded all patients with other types of cancer or who had received radiotherapy or chemotherapy prior to the study. A total of 182 women were included. The cases consisted of 77 newly diagnosed women with a first diagnosis of BC as identified by biopsy. The controls were 105 women with biopsies negative for malignancy who came from the same hospital and geographical area. Written informed consent was obtained from each participant at the time of the interview. The study protocol was approved by the Ethics Committee of the School of Medicine at the University of Coahuila, and the study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Questionnaire application

Information was collected through in-person interviews and included anthropometric parameters, sociodemographic variables (education, socioeconomic status), residence background, reproductive history (age of menarche, age at first pregnancy, number of pregnancies, parity, months of lactation, history of hormonal contraception or replacement therapies, age of menopause), lifestyle factors (smoking, alcohol intake, exercise), a detailed family history of cancer, occupational history, As exposure history and diet.

Blood sample collection

Blood samples (10 mL) were collected from the antecubital vein into 10 mL vacuum blood collection polypropylene tubes (Vacutainer®) with anticoagulant. DNA was obtained from white cells for YAP genotyping and stored at −70°C until analysis.

As measurement in urine

Individual exposure was assessed based on As urinary levels. A first morning void urine sample was collected in sterile 120-mL screw-topped polypropylene containers. Urine samples were analyzed using the methodology described by the U.S. Center for Disease Control (CDC, 2004) at the Arizona Laboratory for Emerging Contaminants, University of Arizona, Tucson, Arizona, U.S.A. In brief, arsenic species in urine [As^{V}, As^{III}, monomethylarsonic acid (MMA^{V}), dimethylarsinic acid (DMA^{V}) and arsenobetaine] were separated by high performance liquid chromatography (HPLC) and analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Arsenic concentrations in urine were analyzed by ICP-MS using standard reference water (SMR 1640 (NIST, Gaithersburg, MD, USA) and freeze-dried urine reference material for trace elements (Clincheck-control; RECIPE Chemicals instruments GmbH, Munich, Germany) in urine as quality controls. Urinary As concentrations were adjusted by urine creatinine levels to account for variations due to urine density. Additional exposure to other arsenic compounds, which is usually attributable to consumption of rice and seafood such as bivalves and seaweeds, was considered minimal because such seafood is rarely eaten in this geographical area. Arsenic

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metabolism efficiency was calculated using the formulas proposed by Del Razo et al. (1997): first methylation = $MMA^{V}/(As^{V}+As^{III})$; second methylation = DMA^{V}/MMA^{V}.

YAP gene polymorphisms (rs11225261 and rs1820453)

DNA extraction was performed by Nucleospin Blood kit (Macherey Nagel®; Dueren, Germany) according to the supplier's instructions. The quantity and quality of DNA was quantified by NanoDrop® spectrophotometer (Wilmington, NC, USA), producing A260/A280 ratios between 1.60 and 1.90. Both procedures were conducted at the Biomedical Research Center, School of Medicine, University of Coahuila, Mexico.

Allelic discrimination of the single nucleotide polymorphisms rs1820453 and rs11225261 of the YAP gene was conducted with the real-time PCR technique, using Taqman Probes genotyping assays (Applied Biosystems®; Foster City, California, USA) and a 7500 Fast Real-Time PCR System (Applied Biosystems®; Foster City, California, USA). In brief, a 10 μL reaction mixture was prepared containing 5 μL of Master Mix (2X) (contains Taq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffer), 0.25 μL of Gene Expression Assay Mix (20X) assay mixture (includes specific primers and predesigned probes with FAM and VIC labels as fluorochromes), 1 μL of sample and 3.5 μL of ultrapure water for PCR. The solution was placed in PCR tubes with cap optical quality. The reaction tubes were placed in a 96-well plate and subjected to the following conditions in a 7500 Fast Real-Time PCR System (Applied Biosystems®; Foster City, California, USA): denaturation of DNA at 95°C for 15 seconds, hybridization of the primers and extension at 60°C for 1 minute (50 cycles).

The primer for the rs11225261 polymorphism was TGCCAGTTTTAGGGGCCATTTGGCT**[C/T]**CTGAGAAGAACT, and the primer for the rs1820453 polymorphism was TCCGTACTTACGAACATGGACTTCT**[T/G]**CGTGATTAAAAAACCCAACGCCGAA. Positive and negative controls were also added in each PCR. For each assay, a genotype was automatically assigned using the Applied Biosystems® TaqMan® Genotyper Software. All assays were performed at the Department of Molecular and Cellular Medicine of the University of Arizona, Tucson, Arizona, USA.

The Hardy-Weinberg equilibrium was tested using a goodness-of-fit Chi-square test to compare the observed genotype frequencies to the expected frequencies among control subjects. With the aim of avoiding bias due to ethnicity, only subjects born in the studied geographical areas were included, and no familial samples were included.

Statistical analysis

Independent and dependent variables were described according to their frequencies and distribution measurements (arithmetic mean and standard deviation). The Chi^2 test or F test was used when the variable was divided into more than two categories. According to the data distribution or to the dichotomous variable, Student's t-test or the Mann-Whitney test was used to compare different YAP genotyping and/or arsenic levels. This method permitted us to establish significant differences among groups for each dependent variable. Linear regression and odds-ratio models were used to assess crude or independent associations

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between breast cancer with the different YAP genotypes and with arsenic metabolite urine concentrations. In all multivariable models, we included those statistically significant variables ($P<0.05$) identified in the bivariate model (smoking, type of drinking water and As metabolites). The variables that produced collinearity in the multivariate analyses were not included in the final models. All analyses were performed using the statistical software STATA 11.0 (Stata Corp., College Station, TX, USA).

Results

A total of 182 women were included in the study and their anthropometric, sociodemographic and lifestyle characteristics are shown in Table 1. Smoking and type of drinking water were significantly different between the studied groups. The cases drink tap water more frequently than controls (Table 1). When the arsenic urinary levels were compared between smokers and non-smokers by cases-controls, no significant differences were found. Urinary arsenic levels were higher (32.7 ± 31.8) in subjects with the lower economic income (22.4 ± 14.9); however, and in contrast, smoking was higher among subjects with the highest income level (38% vs 26%; respectively).

Urinary arsenic levels

Mean total arsenic urinary levels in the entire population were 32.38 ug/L. The correlation between As tap water level with As urinary level was 0.67. When the urinary arsenic concentrations were compared between the studied groups, As^{III}, As^{V}, iAs, DMA^{V}, total As and %iAs, were significantly higher in the cases than in controls, whereas %MMA^{V} and first methylation values were significantly higher in the controls (Table 2).

YAP gene polymorphisms

When the genotypes in the studied population were analyzed, the results revealed that the wild-type genotype frequencies for YAP rs11225261 and YAP rs1820453 polymorphisms were greater than 92% and 46%, respectively. When the heterozygous and homozygous mutant variants were combined, a higher frequency of these variants was observed in subjects with the YAP rs1820453 polymorphism (53.5%), whereas the frequency was lower in the YAP rs11225261 polymorphism carriers (7.1%). No significant differences were found when the YAP polymorphism genotyping frequencies were compared between cases and controls (Table 3). The genotype distributions of the polymorphisms were in accordance with Hardy-Weinberg equilibrium.

Urinary arsenic levels by YAP gene polymorphisms

When the urinary arsenic metabolites levels were compared between cases and controls, the carriers of the wild-type genotype of YAP rs11225261 polymorphism, As^{III}, As^{V}, iAs, total As and second methylation levels were significantly higher in the cases, whereas the first methylation concentration was higher in the controls. In carriers of the combination of heterozygous and homozygous mutant variants (wt/vt+vt/vt) of the YAP rs11225261 polymorphism, the first methylation levels were higher in the controls than in cases (Table 4). In the cases, when the heterozygous and homozygous mutants were combined the carriers of the YAP rs11225261 polymorphism, MMA^{V} and DMA^{V} urinary levels were

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significantly lower than in those cases with the wild-type variant (wt/wt). Additionally, in the controls, no differences were found when the urinary arsenic metabolites were compared by genotyping (Table 4).

When the urinary arsenic metabolites levels were compared between cases and controls, the carriers of the wild-type genotype of YAP rs1820453 polymorphism and the As^{III} and iAs levels were significantly higher in the cases. In carriers of the combination of the heterozygous and homozygous mutant variants, significantly higher levels of As^{V}, iAs and second methylation levels were registered in the cases, and MMA^{V} and first methylation urinary concentrations were higher in the controls (Table 5).

According to genotyping, in the cases, the As^{III}, MMA^{V} and DMA^{V} urinary levels were significantly lower in subjects carrying the combination of heterozygous and homozygous mutant variants of YAP rs1820453 polymorphism compared with the levels observed in the wild-type carriers. No differences were found in the controls when the urinary arsenic metabolites were compared by genotyping (Table 5).

Associations between breast cancer and arsenic urinary levels and YAP gene polymorphisms

When associations between BC and arsenic urinary levels were assessed by bivariate and multivariate linear statistical models, positive and significant associations were found between BC and smoking, type of drinking water, As^{III}, As^{V}, and iAs, whereas a negative and significant association was found with first methylation. A positive association was found with DMA^{V} levels in the bivariate model, but this significant association disappeared in the multivariate model. No significant associations were found between breast cancer and YAP polymorphisms (Table 6).

Risk for breast cancer

In the logistic multivariate statistical model, a significantly increased risk for BC was registered with type of drinking water and As^{V} and iAs levels, and a decreased risk was observed with first methylation (Table 7).

Discussion

Studies of the interactions between genes and the environment are one of the most relevant research lines in cancer etiology. To our knowledge, this study is the first to report interaction between two polymorphisms of YAP, which is a protein that promotes expression of genes involved in cell proliferation and apoptosis suppression and whose dysregulation might contribute to a malignant cellular phenotype, with arsenic levels, which is a widely known carcinogenic metalloid in breast cancer subjects.

A total of 77 women with BC and 105 controls were included in the study. No significant differences were found in almost all sociodemographic variables analyzed, except for smoking and source of drinking water. Increasing evidence highlights that external factors are involved in the development of breast cancer, such as nutrition (obesity and alcohol consumption), smoking, and exposure to carcinogens (e.g., metal compounds) (Jevtic et al.,

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2010). Smoking was significantly higher in cases than in controls (P=0.024), data that support recent evidence that any history of smoking increases the relative risk of breast cancer by approximately 10% (Alberg et al., 2015; IARC, 2012). However, certain studies implicate a potential contribution of arsenic to breast cancer development (Benderli Cihan et al., 2011; Dantzig, 2009; Joo et al., 2009). It is widely known that the most important source of As exposure is drinking water and that chronic exposure to elevated levels of iAs is associated with the development of cancer and other adverse outcomes (NRC, 2001; WHO, 2001). In this study, BC patients more frequently drank tap water than the controls (P=0.009). Higher As urinary levels were detected in the cases as well, and positive associations between BC with selected As metabolites were registered. Similarly, Benderli Cihan et al. (2011), Joo et al. (2009) and Muszy ska et al. (2012) found higher As levels in BC patients compared than in controls, and recently, Lopez-Carrillo et al. (2014) reported an increased BC risk in Mexican women with high %MMA urinary levels. In contrast, an ecological study conducted in Argentina by Aballay et al. (2012) did not report significant association between arsenic concentrations in water and breast cancer. This difference may be due to the fact that Aballay et al. measured exposure for determining As in water levels; whereas, we and the previously mentioned authors quantified exposure in individuals by measuring the internal As dose of the metalloid in urine.

Experimental studies have evaluated As effects on breast tissue and reported that in the rat prepubertal mammary gland, *in utero* exposure to arsenic resulted in an increase in the number of mammosphere-forming cells in branching epithelial cells and the cell density, which resulted in abnormal growth and susceptibility of the gland to neoplasia later in life (Parodi et al., 2015). Arsenic induced the acquisition of a cancer cell phenotype in human breast epithelial cells, resulting in a basal-like phenotype with aromatase overexpression and an increase in breast cancer stem cells, which supports the possibility that arsenic exposure might contribute to the development of an important subset of breast cancers (Xu et al., 2014). Additionally, in a spontaneous tumor mouse model, arsenite in drinking water led to a significant acceleration in growth of mammary tumors (Schrauzer et al., 1978). These data support the hypothesis of As as a promoter and/or contributor to BC development.

The mechanisms of arsenic toxicity are not yet fully understood, and for this reason, several reports have been published that attempt to explain its role in the etiology of BC. Among these mechanisms are the alteration of estrogen receptor (ER) expression and the ability to activate quiescent ER genes (Stoica et al., 2000; Xu et al., 2014). Another important risk factor for BC is inter-individual As metabolism. Altered profiles of As species in urine might determine individual cancer susceptibility. It is known that changes in the capacity to methylate inorganic arsenic modifies the biotransformation and retention of arsenic, with methylated species of arsenic (MMA and DMA) showing less reactivity with tissue constituents (Vahter M, 2002). Recently, Pierce et al. (2019) identified a coding variant in FTCD associated with arsenic metabolism efficiency, providing new evidence supporting the established link between one-carbon/folate metabolism and arsenic toxicity. In our study, a decreased methylation capacity was registered in cases compared with that measured in controls, and positive associations between BC and As^{III}, As^{V} and iAs (a non-methylated As metabolites) were found. Similarly, Pineda-Belmontes et al. (2016) reported increased risk for BC in those females with a high first methylation ratio. The reduction of

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biotransformation from iAs to methylated species recorded in our cases suggests either saturation or inhibition of methylation, which could represent a potential risk factor for BC development and for all the pathways involved in As biotransformation/detoxification, including the Hippo pathway.

Another factor that could be a contributor to cancer genesis is oxidative DNA damage induced by arsenic exposure, and this scenario has been proposed as one of the main mechanisms of arsenic carcinogenicity (Hubaux et al., 2013). Indeed, As exposure and As-induced oxidative and nitrosative stress via the production of reactive oxygen species and reactive nitrogen species have been recognized as a main contributor to the etiology and development of BC (Thomas-Schoemann et al., 2012). Cells have different defense mechanisms against oxidative and nitrosative stress, and among them, the YAP gene is involved in cellular redox environment and in genomic homeostasis. Indeed, YAP plays an important role in the response to endogenous and xenobiotic-induced oxidative stress and supplies resistance to cytotoxic agents and heavy metals (Toone and Jones, 1999). Experimental studies in yeast have reported that when yeast is exposed to oxidants and metalloid exposure, Yap1p transiently accumulates in the nucleus (Delaunay et al., 2000; Kuge et al., 1997, 2001; Yan et al., 1998) and activates transcription of genes coding for proteins that maintain a favorable cellular redox balance (*GSH1*, *TRX2*, *TRR1*, *GLR1*, and *GRE2*) as well as those enzymes involved in detoxification of reactive oxygen species (Gasch et al., 2000; Lee et al., 1999; Toone and Jones, 1999). The induction of genes responsible for synthesis of these detoxification enzymes is largely absent in the Yap1-strain compared with strain yeast. Therefore, the metalloid sensitivity of Yap1-strain can be attributed at least in part to a lack of transcriptional activation of the oxidative stress defense genes (Toone and Jones, 1999), which could contribute to the development of cancer.

With respect to the relationship between genomic homeostasis and As, it is also known that As activates, induces or alters phosphorylation-dependent signaling pathways including the Hippo signaling pathway, which is involved in the control of cell proliferation, adhesion and migration. If this pathway is abnormally activated, induced, or altered, it contributes to the pathogenesis of neoplasms (Alp et al., 2010; Li et al., 2013). In our previous study, we demonstrated that As is able to reduce YAP translocation from the cytoplasm to the nucleus, which can induce a favorable environment for the inhibition of apoptosis, thus promoting cellular proliferation by increasing the genetic instability of cells that might contribute to the pathogenesis of cancer (Michel-Ramirez et al., 2017). In the current study, we did not find associations or an increased risk for BC in those carriers of the studied YAP polymorphisms. No interactions between the YAP polymorphisms and arsenic urinary levels were observed. However, based on our previous results and strong data reported by others, we cannot discard As-YAP interaction as important risk factor for BC, and therefore, further cohort studies are needed to evaluate gene-environment interactions between other YAP genetic polymorphisms with arsenic metabolism. These studies should include a larger sample size and measurement of As urinary species over time with the aim of avoiding over- and underestimates of exposure.

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Conclusions

A positive and significant association was found between breast cancer and smoking, source of drinking water, and As^{III}, As^{V} and iAs levels. Additionally, a negative association with first methylation was observed. A significant increased risk for BC was found with source of drinking water, As^{V}, and iAs, and low risk was noted with first methylation urinary levels. No interactions were recorded between the evaluated YAP gene polymorphisms and arsenic urinary levels.

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Table 1.

Anthropometric/sociodemographic characteristics and lifestyle factors of participants. Results shown as arithmetic mean and standard deviation (percent).

	Cases (n=77)	Controls (n=105)	P-value
Age (years)	52.03 ± 11.27	49.30 ± 10.94	0.101
Body mass index	29.80 ± 5.92	29.03 ± 5.31	0.360
Schooling (years)	9.51 ± 5.54	9.77 ± 4.30	0.730
Age at menarche (years)	12.64 ± 1.43	12.55 ± 1.55	0.668
First childbirth age (years)	21.80 ± 5.23	21.04 ± 4.79	0.327
Number of childbirth	3.96 ± 2.72	3.65 ± 1.56	0.339
Breastfeeding (months)	6.86 ± 6.85	7.72 ± 9.22	0.490
Age at menopause (years)	46.16 ± 5.86	44.76 ± 6.62	0.241
Time living in Comarca Lagunera (years)	44.64 ± 15.83	41.08 ± 15.21	0.465
History of smoking	32 (41.56)	27 (25.71)	**0.024**
History of alcohol intake	14 (18.18)	20 (19.05)	0.882
Living near to an industry	14 (11.69)	17 (16.19)	0.66
Family history of breast cancer	19 (24.68)	20 (19.05)	0.459
Type of drinking water n (%)			
Purified	41 (53.25)	78 (74.29)	**0.009**
Tap water	31 (40.26)	25 (23.81)	
Both	5 (6.49)	2 (1.90)	
Type of water used in cooking n (%)			
Purified	19 (24.68)	30 (28.57)	0.289
Tap water	53 (68.83)	62 (59.05)	
Both	5 (6.49)	13 (12.38)	

[*] P <0.05: Chi^2 test, Student's t–test, or Mann-Whitney test.

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Table 2.

Urinary arsenic concentrations by cases and controls. Results shown as arithmetic mean, standard deviation and percent.

	Cases (n=77)	Controls (n=105)	P-value
Arsenic metabolites (ug/L)			
As^{III}	2.56 ± 3.52	1.64 ± 1.38	**0.016**
As^{V}	2.77 ± 4.26	1.33 ± 2.12	**0.003**
iAs	5.33 ± 6.62	2.97 ± 2.98	**0.001**
MMA^{V}	4.67 ± 8.77	3.80 ± 3.60	0.362
DMA^{V}	29.41 ± 41.37	20.44 ± 17.25	**0.047**
Total As	39.42 ± 53.83	27.23 ± 22.58	**0.038**
%iAs	16.88	11.26	**0.001**
$\%MMA^{V}$	11.22	14.00	**0.0002**
$\%DMA^{V}$	71.88	74.72	0.123
Methylation profile (ug/L)			
First methylation	1.08 ± 0.76	1.83 ± 1.86	**0.001**
Second methylation	10.36 ± 20.08	6.42 ± 4.93	0.054

iAs (inorganic arsenic), MMA^{V} (monomethylarsonic acid), DMA^{V} (dimethylarsinic acid).

[*] P <0.05: Mann-Whitney or Student's t-test.

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Table 3.

Frequency of YAP polymorphism genotyping. Results shown as frequency (percent).

Gene rs#	Genotyping	Cases (n=77)	Controls (n=105)	P-value
YAP				
rs11225261				
	wt/wt	68 (91.89)	89 (93.68)	0.519
	wt/vt	6 (8.11)	5 (5.26)	
	vt/vt	0	1 (1.05)	
	wt/vt + vt/vt	6 (8.11)	6 (6.31)	
rs1820453				
	wt/wt	38 (51.35)	40 (42.55)	0.436
	wt/vt	28 (37.84)	40 (42.55)	
	vt/vt	8 (10.81)	14 (14.89)	
	wt/vt + vt/vt	36 (48.65)	54 (57.44)	

wt = wild type; vt = variant type.

*$P < 0.05$: Chi^2 test.

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Table 4.

Arsenic urinary by YAP rs11225261 polymorphism genotypes and by studied groups. Results are shown as arithmetic mean and standard deviation.

	Cases				Controls			
	wt/wt (n=68)	**wt/vt (n=6)**	**vt/vt (n=0)**	**wt/vt + vt/vt (n=6)**	**wt/wt (n=89)**	**wt/vt (n=5)**	**vt/vt (n=1)**	**wt/vt + vt/vt (n=6)**
Arsenic metabolites								
As^{III}	2.71 ± 3.71 *	1.63 ± 0.84	---	1.63 ± 0.84	1.62 ± 1.35	1.41 ± 1.25	0.94	1.33 ± 1.13
As^{V}	2.76 ± 4.23 *	3.98 ± 5.58	---	3.98 ± 5.58	1.32 ± 2.21	1.03 ± 1.27	0.47	0.93 ± 1.16
iAs	5.47 ± 6.83 *	5.61 ± 5.68	---	5.61 ± 5.68	2.94 ± 3.08	2.44 ± 2.43	1.42	2.27 ± 2.22
MMA^{V}	5.08 ± 9.26	1.58 ± 1.22	---	1.58 ± 1.22 &	3.87 ± 3.76	3.96 ± 2.23	3.36	3.86 ± 2.01
DMA^{V}	31.76 ± 43.42	11.24 ± 8.06	---	11.24 ± 8.06 &	20.97 ± 18.14	23.03 ± 14.53	11.99	21.19 ± 13.75
Total As	42.32 ± 56.6 *	18.44 ± 7.82	---	18.44 ± 7.82	27.80 ± 23.67	29.44 ± 18.96	16.78	27.33 ± 17.73
As methylation profile								
First methylation	1.10 ± 0.74 *	0.81 ± 1.01	---	0.81 ± 1.01 *	1.92 ± 1.97	2.32 ± 0.98	2.36	2.32 ± .88
Second methylation	9.84 ± 20.54 *	18.10 ± 19.78	---	18.10 ± 19.78	6.05 ± 2.67	5.78 ± 1.22	3.56	5.41 ± 1.42

*& P < 0.05: Mann-Whitney test.

* Cases vs. controls: wt/wt vs. wt/wt and wt/vt+vt/vt vs. wt/vt+vt/vt.

& Genotyping in cases or controls: wt/wt vs. wt/vt+vt/vt.

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Table 5.

Urinary arsenic by YAP rs1820453 polymorphism genotypes and by studied groups. Results are shown as arithmetic mean and standard deviation.

	Cases				Controls			
	wt/wt (n=38)	wt/vt (n=28)	vt/vt (n=8)	wt/vt + vt/vt (n=36)	wt/wt (n=40)	wt/vt (n=40)	vt/vt (n=14)	wt/vt + vt/vt (n=54)
Arsenic metabolites								
As^{III}	3.5 ± 4.5*	1.6 ± 1.7	1.6 ± 0.8	1.6 ± 1.5&	1.7 ± 1.5	1.4 ± 1.2	1.6 ± 1.1	1.5 ± 1.1
As^{V}	2.7 ± 3.1	3.5 ± 5.9	0.8 ± 1.6	2.9 ± 5.3*	1.8 ± 3.0	0.7 ± 0.8	1.2 ± 1.4	0.8 ± 1.0
iAs	6.3 ± 7.1*	5.1 ± 6.9	2.5 ± 1.9	4.5 ± 6.2*	3.6 ± 4.0	2.1 ± 1.7	2.9 ± 2.3	2.3 ± 1.9
MMA^{V}	6.9 ± 12.0	2.4 ± 1.9	2.6 ± 1.2	2.4 ± 1.7*&	4.1 ± 4.6	0.6 ± 2.8	3.9 ± 2.4	3.7 ± 2.7
DMA^{V}	40.9 ± 55.5	17.2 ± 12.5	23.3 ± 11.5	18.6 ± 12.4&	23.7 ± 22.4	18.2 ± 14.2	21.7 ± 11.0	19.1 ± 13.5
Total As	54.3 ± 72.4	24.8 ± 16.2	28.5 ± 13.9	25.6 ± 15.6	31.4 ± 29.6	24.1 ± 17.9	28.6 ± 14.8	25.2 ± 17.1
As methylation profile (ug/L)								
First methylation	1.2 ± 0.7	0.8 ± 0.8	1.2 ± 0.5	0.9 ± 0.7*	1.5 ± 1.0	2.4 ± 2.6	1.7 ± 0.8	2.2 ± 2.3
Second methylation	6.8 ± 3.6	15.8 ± 32.6	9.1 ± 2.7	14.3 ± 28.8*	6.5 ± 2.9	5.3 ± 2.0	6.0 ± 2.7	5.5 ± 2.2

*& $P < 0.05$: Mann-Whitney test.

* Cases vs. controls: wt/wt vs. wt/wt and wt/vt+vt/vt vs. wt/vt+vt/vt.

& Genotyping in cases or in controls: wt/wt vs. wt/vt+vt/vt.

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Table 6.

Crude and adjusted linear regression between breast cancer and lifestyle factors, arsenic urinary levels, and YAP polymorphisms.

	β		95% CI		P-value	
	Crude	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**
Lifestyle factors						
Smoking	0.176	0.147	0.02 0.32	0.00 0.29	**0.024**	**0.047**
Type of drinking water	0.199	0.151	0.07 0.32	0.02 0.27	**0.002**	**0.020**
Urinary arsenic metabolites						
As^{III}	0.034	0.02	0.00 0.06	0.00 0.05	**0.017**	**0.049**
As^{V}	0.032	0.02	0.01 0.05	0.00 0.04	**0.003**	**0.008**
iAs	0.023	0.01	0.00 0.03	0.00 0.03	**0.001**	**0.006**
MMA^{V}	0.005	0.002	−0.00 0.01	−0.00 0.01	0.362	0.623
DMA^{V}	0.002	0.001	0.00 0.00	−0.00 0.00	**0.047**	0.17
Total As	0.001	0.001	0.00 0.00	−0.00 0.00	0.038	0.142
As methylation profile						
First methylation	−0.077	−0.07	−0.12 −0.03	−0.11 −0.02	**0.001**	**0.001**
Second methylation	0.005	0.005	−0.00 0.01	−0.00 0.01	0.054	0.054
YAP polymorphism						
rs11225261	0.021	0.096	−0.24 0.28	−0.19 0.38	0.869	0.509
rs1820453	−0.065	−0.08	−0.17 0.04	−0.23 0.06	0.236	0.249

Adjusted by smoking, type of drinking water, As^{III}, As^{V}, iAs, DMA^{V}, total As and first methylation.

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Table 7.

Odds ratio (OR) for breast cancer and lifestyle factors, arsenic urinary levels and YAP polymorphism.

	OR		95% CI		P-value	
	Crude	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**
Lifestyle factors						
Smoking	2.05	1.87	1.09 3.85	0.94 3.74	**0.025**	0.073
Type of drinking water	2.29	1.88	1.32 3.97	1.03 3.44	**0.003**	**0.039**
Urinary arsenic metabolites						
As^{III}	1.18	1.15	1.01 1.38	0.98 1.34	**0.031**	0.07
As^{V}	1.18	1.16	1.04 1.33	1.02 1.31	**0.01**	**0.02**
iAs	1.12	1.10	1.03 1.22	1.01 1.20	**0.005**	**0.015**
MMA^{V}	1.02	1.01	0.97 1.07	0.96 1.06	0.38	0.62
DMA^{V}	1.01	1.00	0.99 1.02	0.99 1.02	0.07	0.20
Total As	1.00	1.00	0.99 1.02	0.99 1.01	0.06	0.17
As methylation profile						
First methylation	0.47	0.48	0.31 0.70	0.32 0.74	**0.000**	**0.001**
Second methylation	1.06	1.06	0.99 1.12	0.99 1.13	0.071	0.059
YAP polymorphisms						
rs11225261	1.30	1.50	0.40 4.23	0.44 5.04	0.65	0.50
rs1820453	0.70	0.68	0.38 1.29	0.36 1.30	0.25	0.25

Adjusted by smoking, type of drinking water, As^{III}, As^{V}, iAs, DMA^{V}, total As and first methylation.