Published in final edited form as:

Environ Sci Technol. 2018 December 18; 52(24): 14487–14495. doi:10.1021/acs.est.8b06457.

Circulating miRNAs associated with arsenic exposure

Rowan Beck^{1,2}, Paige Bommarito³, Christelle Douillet⁴, Matt Kanke⁵, Luz M Del Razo⁶, Gonzalo García-Vargas⁷, Rebecca C. Fry³, Praveen Sethupathy^{5,*}, Miroslav Styblo^{4,*}

¹Department of Genetics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA.

²Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA.

³Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, North Carolina, USA.

⁴Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, USA.

⁵Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York.

⁶Department of Toxicology, Center of Investigation and of Advanced Studies of the National Polytechnic Institute (Cinvestav-IPN), México City, Mexico

⁷Faculty of Medicine, Juarez University of Durango State, Durango, Mexico

Abstract

Arsenic (As) is a toxic metalloid. Inorganic arsenic (iAs) is a form of As commonly found in drinking water and in some foods. Overwhelming evidence suggest that people chronically exposed to iAs are at risk of developing cancer, or cardiovascular, neurological and metabolic diseases. Although the mechanisms underlying iAs-associated illness remain poorly characterized, a growing body of literature raises the possibility that microRNAs (miRNAs), post-transcriptional gene suppressors, may serve as mediators and/or early indicators of the pathologies associated with iAs exposure.

To characterize the circulating miRNA profiles of individuals chronically exposed to iAs, samples of plasma were collected from 109 healthy residents of the city of Zimapán and the Lagunera area in Mexico, the regions with historically high exposures to iAs in drinking water. These plasma samples were analyzed for small RNAs using high-throughput sequencing, and for iAs and its methylated metabolites. Associations between plasma levels of arsenic species and miRNAs were evaluated.

Six circulating miRNAs (miRs-423–5p, –142–5p –2, –423–5p +1, –320c-1, –320c-2, and –454–5p), two of which have been previously linked to cardiovascular disease and diabetes (miRs-423–5p, –454–5p), were found to be significantly correlated with plasma MAs. No miRNAs were associated with plasma iAs or DMAs after correction for multiple testing. These miRNAs may

* Corresponding Authors: Praveen Sethupathy, pr46@cornell.edu; Miroslav Styblo, styblo@med.unc.edu.

represent mechanistic links between iAs exposure and disease or serve as markers of disease risks associated with this exposure.

Introduction

Inorganic arsenic (iAs) is a common drinking water contaminant. Exposure to iAs through consumption of contaminated water is of concern for over 200 million people in 70 countries, including the United States, Mexico, Bangladesh, or China¹. These individuals are exposed to levels of iAs higher than the maximum limit of 10 µg As/L (10 ppb) set by the US EPA and World Health Organization². Though exposure to iAs in drinking water is the primary concern, additional exposures are associated with the consumption of foods grown in iAs-rich soils¹. Because iAs is a group 1 human carcinogen³, the US EPA and the Agency for Toxic Substances and Disease Registry (ATSDR) rank arsenic (As) as first on the US Priority List of Hazardous Substances². The association between exposure to iAs in drinking water and cancer, including cancer of the urinary bladder, kidney, skin, and liver has been firmly established³. More recent and growing evidence has linked iAs exposure also to non-cancer pathologies, for example cardiovascular disease^{4,5}, hypertension^{6,7}, and diabetes^{8–10}.

Mexico is among the countries where environmental iAs exposure is of particular concern because of rich mineral deposits, which lead to high levels of naturally occurring iAs^{11–14}. The contamination of drinking water supplies with iAs has been documented in 14 Mexican states: Zimapan, Durango, Coahuila, Zacatecas, Morelos, Aguascalientes, Chihuahua, Sonora, Puebla, Nuevo León, Jalisco, Oaxaca, Guanajuato and San Luis Potosí¹⁵. An estimated 450,000 residents of these states use drinking water supplies, in which iAs concentrations exceed 50 µg As/L¹⁶. Adverse health effects associated with iAs exposure in these regions include skin lesions¹⁷, increased risk of cardiometabolic diseases¹⁸, including diabetes mellitus¹⁹, and neurological²⁰ and genotoxic effects²¹. The present study used samples and data that were collected in Zimapan and in the Lagunera region, an area located on the border of the Durango and Coahuila states.

The individual susceptibility to adverse effects of iAs exposures is determined by a variety of factors, most importantly by the efficiency of iAs metabolism²². Humans, as well as many animal species, metabolize iAs in a series of methylation reactions that are catalyzed by a single enzyme arsenic (+3 oxidation state) methyltransferase (AS3MT) to form monomethyl-As (MAs) and dimethyl-As (DMAs) metabolites, which are excreted primarily in the urine. The urinary profiles of As species have often been used in population studies to characterize both iAs exposure and the pattern of iAs metabolism. High proportions of urinary DMAs (%DMAs) and low proportions of iAs (%iAs) and MAs (%MAs) in the urine are generally considered indicators of efficient iAs metabolism, whereas low %DMAs or low DMAs/MAs ratio is thought to indicate an impairment in iAs methylation. Analysis of iAs and its metabolites in other biological specimens, e.g., blood, plasma, saliva, or exfoliated epithelial cells, although more difficult than in urine, have been used in some studies to characterize the internal dose of As in iAs-exposed individuals^{23–26}. While the

role of iAs metabolism in the adverse effects of iAs exposure is well characterized, less is known about molecular factors that mark and/or underlie these effects.

There has been a growing interest among researchers to identify sensitive, non-invasive molecular markers to aid in disease risk prediction. MicroRNAs (miRNAs) have emerged as potential early markers of disease risk, and in some instances, mediators of disease²⁷. MiRNAs are small RNAs consisting of ~22 nucleotides, that regulate gene expression by binding to the 3'-untranslated regions of one or more target mRNAs²⁸. They have emerged as important components of gene regulation that control a wide array of physiological and pathological processes, most notably cancer development²⁹, hypertension³⁰, atherogenesis³¹, insulin resistance³², and overt type 2 diabetes etiology³³.

MiRNAs are also present and highly stable in many different extracellular fluids, including blood, urine, and saliva. Most miRNAs are fairly widespread in expression and so it is often difficult at best to discern the tissue-of-origin for specific miRNAs altered in circulation³⁴. However, some miRNAs tend to be robustly enriched in a few cell or tissue types; examples include miR-122 in liver, miR-206 in muscle, and miR-514a-3p in testis³⁵. If such miRNAs are present in circulation at aberrant levels then it could be indicative of dysregulation in specific tissues³⁶. There is growing interest in the potential of circulating miRNAs to serve as early markers before overt disease manifestation, as well as prognostic indicators of disease course³⁷. Several recent and ongoing clinical trials have examined the use of specific miRNAs as predictors of disease, including miR-29b in blood and saliva as a prognostic measure for oral squamous cell carcinoma, and 10b as a circulating biomarker of gliomas³⁸.

MiRNAs in tissues such as liver³⁹ and adipose^{40,41} have been shown to respond to various metabolic and environmental stimuli, including exposure to iAs^{42–44}. Notably, some of the miRNAs that were found to be altered by iAs exposure have also been found to play a role in etiology of various human diseases, e.g., cancer or diabetes. The goal of the present study was to identify circulating plasma miRNAs that are characteristic of chronic iAs exposure as the first step in determining the role of miRNA as potential mediators or markers of disease associated with exposure.

Materials and Methods

Study participants and sample collection.

The present study used samples and data collected previously from participants in the Zimapán and Lagunera cohorts in Mexico¹⁹. These cohorts were described in detail elsewhere¹⁹. Briefly, the participants (males and females, 5 years old) were recruited among residents of the Zimapán and Lagunera regions who were exposed to iAs in drinking water. Only participants with two or more years of residency in the study areas were recruited. Excluded from the study were pregnant women and individuals who reported alcohol abuse or having a chronic or acute urinary tract disease or type I diabetes. All procedures included in this study were approved by Institutional Review Boards of Cinvestav-IPN, Mexico City, Mexico and University of North Carolina at Chapel Hill, North Carolina, USA. Each of the 258 participants underwent a medical exam and provided samples of fasted venous blood from which plasma was prepared by centrifugation at 4°C.

All participants also provided samples of water they typically used for drinking. In these cohorts, iAs exposure was associated with increased prevalence of diabetes¹⁹. The present study used plasma samples from 109 randomly selected participants that were not diagnosed with diabetes or pre-diabetes.

Analyses of As and As species in water and plasma

The analysis of As in drinking water was described in detail in the original report on the Zimapán and Lagunera cohort¹⁹. Plasma samples were analyzed in the Styblo lab at the University of North Carolina, Chapel Hill using hydride generation–cryotrapping (HG-CT)-inductively coupled plasma-mass spectrometry (ICP-MS). This method, including sample preparation, limits of detection, coefficients of variation, and procedures used for quality assurance/control, have been described in detail in our previous reports^{45,46}. Using HG-CT-ICP-MS, we measured concentrations of iAs, MAs, and DMAs in plasma. Total As (TAs) concentration was calculated as the sum of iAs, MAs, and DMAs. The percentages of TAs represented by iAs, MAs, and DMAs (%iAs, %MAs, and %DMAs) were used as markers of the efficiency of iAs metabolism.

High throughput sequencing for miRNA profiling.

Qiagen miRNeasy Serum/Plasma Kit (Qiagen, Venlo, Netherlands) was used to extract RNA from 50 µL aliquots of 109 fasting plasma samples. RNA concentrations ranged from 11 – 140 ng/µL. A Nanodrop spectrophotometer was used to examine RNA concentrations and purity. We performed small RNA sequencing as described previously⁴⁷, using Illumina HiSeq, which resulted in an average of at least 12 million reads per sample.

An alignment tool, miRquant 2.0⁴⁸, was used for adapter trimming, and alignment of reads to the hg19 version of the human genome using two different mapping tools (Bowtie and SHRiMP), before quantifying miRNAs and miRNA isoforms (termed isomiRs). We then performed normalization (reads per million mapped to miRNAs (RPMMM)), and miRNAs with greater than 50 RPMMs in at least one sample were included for further analysis. This threshold yielded a set of 596 detected miRNAs across all samples. MiRNAs with an average <25 RPMMM across all samples were removed which resulted in 362 robustly expressed miRNAs.

Of the 109 samples sequenced, several showed signs of RNA degradation (Supplementary Figure 1) and were excluded from the study. These samples were collected over 10 years ago in remote regions of central and northern Mexico. At these locations, we could not use liquid nitrogen to snap-freeze the plasma samples before transport to Mexico City. Instead dry ice was used. Thus, the observed variances in mapping rates may be attributed to the degradation of RNA due to sample handling. Several other samples exhibited low numbers of mapped reads. Only samples with at least 1 million mapped reads to miRNAs (a total of 82 samples) were used for further analysis.

Statistical analysis.

Variables were assessed using a Kolmogorov–Smirnov test for normality. All normalized sequencing reads (RPMMM) were then transformed with a Box-Cox transformation.

(Package ‘car’; RStudio Version 1.0.136). The association between plasma As levels and miRNA expression levels were evaluated in samples using a general linear regression model, in which log-transformed plasma As was the independent variable and plasma miRNA expression was the dependent variable. Covariates potentially influencing miRNA expression levels such as age, sex, body mass index (BMI), smoking status, and alcohol consumption status were included in the regression model. Significant associations between miRNA expression and plasma As were classified as having a P-value < 0.05 and a false discovery rate (FDR) adjusted value (q-value) < 0.15 using an analysis of covariance (ANCOVA) model. A significance cut-off of $q < 0.15$ was chosen based on previously published circulating miRNA literature^{49–51}.

We computed adjusted R^2 values (R_a^2) using simple linear regression models to estimate the explained variation of the model (i.e., R^2 values were adjusted for the number of parameters in the model).

Results

Basic characteristics of the sub-cohort.

The present study focused on analysis of 82 plasma samples that satisfied criteria for good quality RNA and for number of mapped miRNA reads. The basic demographic characteristics of the donors of these samples are shown in Table 1. The sub-cohort consists mostly of females (73.2%), aged 5–65 years. The majority of participants did not smoke or consume alcohol. BMI ranged from 14.5 to 50 Kg/m²; 62% of participants were obese or overweight with 28% having a BMI > 30 Kg/m². These demographics are fairly consistent with those of the original cohort (N=258)¹³. The original cohort was also comprised of mostly women (67%), and 34% of donors had BMI > 30 Kg/m².

Concentrations of As in water and concentrations of As species in plasma.

Of the 82 individuals, 61 (74.4%) drank water with As levels higher than 10 µg/L (ppb), ranging between 10.3 – 215.2 µg/L (Table 2). The concentrations of TAs in plasma (i.e., sum of iAs+MAs+DMAs) ranged from 0.221 – 9.669 ng/mL. Using a multiple regression analysis adjusted for the number of predictors or independent variables, we observed a modest but statistically significant correlation ($R_a^2 = 0.146$, $P = 0.0002$) between drinking water As and TAs in plasma (Fig 1D). Similar correlations were observed between water As and plasma MAs ($R_a^2 = 0.185$, $P = 0.0001$) or DMAs ($R_a^2 = 0.184$, $P = 0.0001$) (Figure 1B, C). We found no statistically significant correlation between As concentration in water and the concentration of iAs in plasma (Figure 1A).

MiRNA sequencing data.

The top 20 most abundant miRNAs (Figure 2) constitute nearly 80% of reads across all samples, with one miRNA (miR-486–5p) accounting for 35% of reads. This result is concordant with previously published sequencing studies carried out in human plasma. For example, Tong et al reported that in their samples miR-486–5p accounts for nearly 60% of the total number of reads, and 10 miRNAs constitute ~90% of all reads⁵².

MiRNAs associated with As species in plasma.

MiRNAs associated with TAs and or with individual As species in plasma were identified using a linear mixed model, with adjustments for age, sex, BMI, smoking status and alcohol consumption. To conduct a comparative analysis, we used an unadjusted p-value (P) < 0.05 for initial exploration, which revealed 40 shared miRNAs associated with MAs, DMAs, and TAs (Figure 3A). The inclusion of iAs in this analysis reduced the total number of shared miRNAs to 2 with P < 0.05 (Figure 3B). MiRNAs associated with iAs can be distinguished from the miRNAs associated with methylated arsenicals, demonstrating unique characteristics of each arsenical and suggesting they may be targeting different pathways and have different mechanisms contributing to their effects. A list of miRNAs associated with each arsenical can be found in Supplementary Table 2.

Next, P-values were adjusted using a FDR method. Using an adjusted value (q-value) threshold of 0.15, as has been used by other similar circulating miRNA studies^{49–51}, greatly reduced the number of miRNAs associated with each metabolite. Statistically significant associations were found only with 6 miRNAs, and all six were associated with MAs (Figure 4A, 4B; Table 3).

Discussion

The exposure to iAs is of grave concern as it has been associated with a variety of diseases, including cancer³, cardiovascular disease^{18,53}, and diabetes^{9,10,54}. The exact molecular mechanisms underlying these adverse health effects remain largely unknown, though many mechanisms have been proposed, e.g., oxidative stress^{55,56}, altered DNA repair⁵⁷ or DNA methylation and gene expression^{10,58,59}, or endocrine disruption⁹.

Hou et al, 2011 proposed that exposure to chemicals in the environment could alter miRNAs abundance, and that this effect is associated with induction of oxidative stress or inflammation by these chemicals⁶⁰. Sturchio et al then confirmed this by showing that short exposure (24 hrs) to a low concentration of iAs (2 μ M) was enough to alter miRNA expression in Jurkat cells⁶¹. Since then, several studies, including population studies, have observed altered miRNA expression in response to iAs exposure¹⁰. For example, in humans, prenatal iAs exposure altered miRNA and gene expression in cord blood of newborns³⁷. However, to our best knowledge, a large-scale analysis of the associations between iAs exposure and metabolism in adult populations with circulating miRNAs has not been previously published. The present study investigated the relationships between iAs exposure and circulating miRNA using data on iAs in drinking water and plasma samples collected in our previously established cohort in Mexico. The range of iAs concentrations in drinking water used by the participants in this cohort (3.1 to 215.2 μ g/L) resembles those reported from other iAs-exposure areas, including several regions in the United States. For instance, in North Carolina, of 63,000 private wells tested for As, 7712 (12%) contained As at levels ranging from 1 to 806 μ g/L; 1436 wells had As levels above the US EPA limit⁶².

It has been recognized that measures of As in drinking water provide an incomplete picture of iAs exposure because additional exposures occur through diet, particularly consumption of crops grown in iAs-rich soils or foods cooked in water contaminated with iAs. To account

for these sources of exposure, many published studies have measured As species in urine. It is not clear, however, whether urinary As levels and speciation reflect those in circulation or in target tissues. Bommarito and coworkers have recently compared the urinary and plasma concentrations of As species in the entire Zimapán/Lagunera cohort. They found statistically significant positive correlations between TAs, MAs and DMAs, but not iAs, in plasma and urine⁶³. Strong positive correlations were also found between As concentrations in drinking water and plasma TAs, MAs and DMAs. We were able to reproduce their findings in a subset of the cohort in the present study. Taken together, these data suggest that plasma TAs is a reliable indicator of iAs exposure, but the profiles of As species in urine differ from those in circulation.

Using unadjusted p values, we found 2 plasma miRNAs (miRs-30c-1-5p, and -486-5p +1) to be associated with all As measures in plasma, including iAs, MAs, DMAs, and TAs, and a shared 40 miRNAs overlapping between MAs, DMAs and TAs. P-values were FDR adjusted to correct for multiple testing and a significance cut-off of $Q < 0.15$ was chosen based on previously published circulating miRNA literature^{49–51}. Using Q values narrowed the number of associated miRNAs to 6, and all were associated only with plasma MAs. MAs is an intermediary metabolite in the pathway for methylation of iAs⁶⁴. We and others have shown that the trivalent form of MAs (MAs^{III}) is the most toxic among the metabolites of iAs^{65,66}. Notably, we have also shown that MAs^{III} inhibits insulin secretion in murine pancreatic islets⁶⁷, and is also a potent inhibitor of insulin-dependent glucose uptake in adipocytes⁶⁸ and insulin-stimulated glycogen synthesis in hepatocytes⁶⁹. MAs in urine have been linked to adverse effects of iAs exposure in humans⁷⁰. For example, a study by Melak and co-workers indicated that higher proportions of urinary As present in the form of MAs (%MAs) were associated with increased risk of bladder cancer among residents of northern Chile who were exposed to iAs in drinking water⁷¹. Similarly, higher urinary MAs% was risk factor cardiovascular disease in a prospective study in Bangladesh⁷². In contrast, lower %MAs was associated with a higher incidence of diabetes in the Strong Heart study⁷³. Thus, the urinary profiles of As species, MAs in particular, are indicators of disease risk associated with iAs exposure.

Among the six miRNAs identified in this study to be significantly associated with plasma MAs, two were variants of canonical miRNAs, or isomiRs. Two of the six small RNAs identified in the present study have been previously recognized as being associated with diabetes or cardiovascular dysfunction, disorders that have been previously linked with iAs exposure^{9,18}. Our study revealed both miR-423 as well as an isomiR, 423-5p +1, as being significantly associated with plasma MAs. MiR-423-5p has been linked to type 2 diabetes status in patients⁷⁴ and contributed to explain variance in fasting glucose. This connection may be explained by the interaction of NFE2 and miR-423 to repress the FAM3A-ATP-Akt pathway, which promotes gluconeogenesis and lipid deposition in the liver⁷⁵. However, miR-423 is expressed in other tissues as well, including pancreatic islets, and therefore a definitive connection with disease etiology cannot be made without further experimentation. Nabiałek et al. also detected an early rise in miR-423-5p in circulation of patients with acute myocardial infarction⁷⁶, suggesting it may harbor diagnostic potential.

In a study comparing the circulating miRNA profiles of individuals diagnosed with type 1, type 2, or gestational diabetes in Brazil, miR-142-5p was found to be one of nine miRNAs in common with all three types⁷⁷. MiR-142 is also one of four miRNAs that can differentiate patients with childhood dilated cardiomyopathy from healthy subjects⁷⁸. Our study revealed an isomiR, miR-142-5p -2, to be significantly associated with plasma MAs. Finally, it has been shown that miR-454 exhibits altered expression in human cord blood from prenatal infants with maternal exposure to iAs³⁷, and was observed to be downregulated in instances of diastolic dysfunction⁷⁹. These 3 miRNAs or isomiRs have been linked to cardiometabolic disease in the general population as well as to plasma MAs levels in the present study, which suggests that they are not specific to iAs exposure, but rather may serve as indicators of a disease risk in populations exposed to iAs.

While the remaining two miRNAs, (miR-320c-1, -320c-2) have not yet been implicated as circulating factors associated with either diabetes or iAs exposure, miR-320c has been shown to be one of several miRNAs upregulated in the urinary exosomes of patients with type II diabetic nephropathy⁸⁰.

In conclusion, our results suggest that iAs exposure is associated with altered profiles of circulating miRNAs. We have identified 6 miRNAs in plasma that are associated with iAs exposure, specifically with plasma concentration of MAs, a toxic metabolite of iAs. Given the published data, four of these miRNAs (miRs-423-5p, 423-5p +1, -142-5p -2, and -454-5p) appear to be linked to risk of cardiometabolic diseases, while two other miRNAs (-320c-1, and -320c-2) may be specific markers of iAs exposure. It is important to note that these 6 miRNAs were associated with MAs in plasma of healthy individuals. Future studies in large, well-phenotyped populations exposed to iAs will help to further evaluate the sensitivity and specificity of these associations, which will be important for determining the utility of these miRNAs as early biomarkers of iAs exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was funded by a Gillings Innovation Laboratory grant to MS and PS and was also supported by DK 056350 grant to the Nutrition Obesity Research Center at UNC. We are grateful to Zhao Lai at the University of Texas Health Science Center at San Antonio, TX, for small RNA sequencing, and Ben Keith (UNC) for assistance with small RNA sequencing analysis.

References

- (1). Stanton BA; Caldwell K; Congdon CB; Disney J; Donahue M; Ferguson E; Flemings E; Golden M; Guerinot M.Lou ; Highman J; et al. MDI Biological Laboratory Arsenic Summit: Approaches to Limiting Human Exposure to Arsenic. *Curr. Environ. Heal. reports* 2015, 2 (3), 329–337. 10.1007/s40572-015-0057-9.
- (2). Agency for Toxic Substances, D. R. Toxicological Profile for Arsenic. U.S. Department of Health and Human Services. <http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=22&tid=3>.
- (3). International Agency for Research on Cancer (IARC, . Arsenic in Drinking Water. *Int. agency Res. cancer Monogr. Eval. Carcinog. risk to humans Some Drink. water Disinfect. Contam. Incl. Arsen. Lyon vol pp* 2004, 84 SRC-, 269–477.

- (4). Tseng CH; Chong CK; Chen CJ; Tai TY Dose-Response Relationship between Peripheral Vascular Disease and Ingested Inorganic Arsenic among Residents in Blackfoot Disease Endemic Villages in Taiwan. *Atherosclerosis* 1996, 120 (1–2), 125–133. [PubMed: 8645353]
- (5). Chiou H-Y; Huang W-I; Su C-L; Chang S-F; Hsu Y-H; Chen C-J Dose-Response Relationship Between Prevalence of Cerebrovascular Disease and Ingested Inorganic Arsenic. *Stroke* 1997, 28 (9).
- (6). Chen CJ; Hsueh YM; Lai MS; Shyu MP; Chen SY; Wu MM; Kuo TL; Tai TY Increased Prevalence of Hypertension and Long-Term Arsenic Exposure. *Hypertens. (Dallas, Tex. 1979)* 1995, 25 (1), 53–60.
- (7). Rahman M; Tondel M; Ahmad SA; Chowdhury IA; Faruquee MH; Axelson O Hypertension and Arsenic Exposure in Bangladesh. *Hypertens. (Dallas, Tex. 1979)* 1999, 33 (1), 74–78.
- (8). Tseng C-H Metabolism of Inorganic Arsenic and Non-Cancerous Health Hazards Associated with Chronic Exposure in Humans. *J. Environ. Biol* 2007, 28 (2 Suppl), 349–357. [PubMed: 17929750]
- (9). Maull EA; Ahsan H; Edwards J; Longnecker MP; Navas-Acien A; Pi J; Silbergeld EK; Styblo M; Tseng C-H; Thayer KA; et al. Evaluation of the Association between Arsenic and Diabetes: A National Toxicology Program Workshop Review. *Environ. Health Perspect* 2012, 120 (12), 1658–1670. 10.1289/ehp.1104579. [PubMed: 22889723]
- (10). Beck R; Styblo M; Sethupathy P Arsenic Exposure and Type 2 Diabetes: MicroRNAs as Mechanistic Links? *Curr. Diab. Rep* 2017, 17 (3), 18 10.1007/s11892-017-0845-8. [PubMed: 28275977]
- (11). Del Razo LM; Arellano MA; Cebrián ME The Oxidation States of Arsenic in Well-Water from a Chronic Arsenicism Area of Northern Mexico. *Environ. Pollut* 1990, 64 (2), 143–153. 10.1016/0269-7491(90)90111-O. [PubMed: 15092299]
- (12). Del Razo L.; García-Vargas G.; García-Salcedo J; Sanmiguel M.; Rivera M; Hernandez M.; Cebrian M. Arsenic Levels in Cooked Food and Assessment of Adult Dietary Intake of Arsenic in the Region Lagunera, Mexico. *Food Chem. Toxicol* 2002, 40 (10), 1423–1431. 10.1016/S0278-6915(02)00074-1. [PubMed: 12387304]
- (13). Del Razo LM; Corona JC; García-Vargas G; Albores A; Cebrián ME Fluoride Levels in Well-Water from a Chronic Arsenicism Area of Northern Mexico. *Environ. Pollut* 1993, 80 (1), 91–94. 10.1016/0269-7491(93)90015-G. [PubMed: 15091878]
- (14). Armienta MA; Villaseñor G; Rodríguez R; Ongley LK; Mango H The Role of Arsenic-Bearing Rocks in Groundwater Pollution at Zimapán Valley, México. *Environ. Geol* 2001, 40 (4–5), 571–581. 10.1007/s002540000220.
- (15). Bundschuh J; Litter MI; Parvez F; Román-Ross G; Nicolli HB; Jean J-S; Liu C-W; López D; Armienta MA; Guilherme LRG; et al. One Century of Arsenic Exposure in Latin America: A Review of History and Occurrence from 14 Countries. *Sci. Total Environ* 2012, 429, 2–35. 10.1016/j.scitotenv.2011.06.024. [PubMed: 21959248]
- (16). Limón-Pacheco JH; Jiménez-Córdova MI; Cárdenas-González M; Sánchez Retana IM; Gonsébat ME; Del Razo LM Potential Co-Exposure to Arsenic and Fluoride and Biomonitoring Equivalents for Mexican Children. *Ann. Glob. Heal* 2018, 84 (2), 257–273. 10.29024/aogh.913.
- (17). Valenzuela OL; Drobná Z; Hernández-Castellanos E; Sánchez-Peña LC; García-Vargas GG; Borja-Aburto VH; Styblo M; Del Razo LM Association of AS3MT Polymorphisms and the Risk of Premalignant Arsenic Skin Lesions. *Toxicol. Appl. Pharmacol* 2009, 239 (2), 200–207. 10.1016/j.taap.2009.06.007. [PubMed: 19538983]
- (18). Mendez MA; González-Horta C; Sánchez-Ramírez B; Ballinas-Casarrubias L; Cerón RH; Morales DV; Baeza Terrazas FA; Ishida MC; Gutiérrez-Torres DS; Saunders RJ; et al. Chronic Exposure to Arsenic and Markers of Cardiometabolic Risk: A Cross-Sectional Study in Chihuahua, Mexico. *Environ. Health Perspect* 2016, 124 (1), 104–111. 10.1289/ehp.1408742. [PubMed: 26068977]
- (19). Del Razo LM; García-Vargas GG; Valenzuela OL; Castellanos EH; Sánchez-Peña LC; Currier JM; Drobná Z; Loomis D; Styblo M Exposure to Arsenic in Drinking Water Is Associated with Increased Prevalence of Diabetes: A Cross-Sectional Study in the Zimapán and Lagunera Regions in Mexico. *Environ. Heal* 2011, 10 (1), 73 10.1186/1476-069X-10-73.

- (20). Rosado JL; Ronquillo D; Kordas K; Rojas O; Alatorre J; Lopez P; Garcia-Vargas G; Caamaño M del C.; Cebrián, M. E.; Stoltzfus, R. J. Arsenic Exposure and Cognitive Performance in Mexican Schoolchildren. *Environ. Health Perspect* 2007, 115 (9), 1371–1375. 10.1289/ehp.9961. [PubMed: 17805430]
- (21). Méndez-Gómez J; García-Vargas G-G; López-Carrillo L; Calderón-Aranda E-S; Gómez A; Vera E; Valverde M; Cebrián ME; Rojas E Genotoxic Effects of Environmental Exposure to Arsenic and Lead on Children in Region Lagunera, Mexico. *Ann. N. Y. Acad. Sci* 2008, 1140 (1), 358–367. 10.1196/annals.1454.027. [PubMed: 18991935]
- (22). Jansen RJ; Argos M; Tong L; Li J; Rakibuz-Zaman M; Islam MT; Slavkovich V; Ahmed A; Navas-Acien A; Parvez F; et al. Determinants and Consequences of Arsenic Metabolism Efficiency among 4,794 Individuals: Demographics, Lifestyle, Genetics, and Toxicity. *Cancer Epidemiol. Biomarkers Prev* 2016, 25 (2), 381–390. 10.1158/1055-9965.EPI-15-0718. [PubMed: 26677206]
- (23). Currier JM; Ishida MC; González-Horta C; Sánchez-Ramírez B; Ballinas-Casarrubias L; Gutiérrez-Torres DS; Cerón RH; Morales DV; Terrazas FAB; Del Razo LM; et al. Associations between Arsenic Species in Exfoliated Urothelial Cells and Prevalence of Diabetes among Residents of Chihuahua, Mexico. *Environ. Health Perspect* 2014, 122 (10), 1088–1094. 10.1289/ehp.1307756. [PubMed: 25000461]
- (24). Grau-Perez M; Kuo C-C; Gribble MO; Balakrishnan P; Jones Spratlen M; Vaidya D; Francesconi KA; Goessler W; Guallar E; Silbergeld EK; et al. Association of Low-Moderate Arsenic Exposure and Arsenic Metabolism with Incident Diabetes and Insulin Resistance in the Strong Heart Family Study. *Environ. Health Perspect* 2017, 125 (12). 10.1289/EHP2566.
- (25). Howe CG; Liu X; Hall MN; Slavkovich V; Ilievski V; Parvez F; Siddique AB; Shahriar H; Uddin MN; Islam T; et al. Associations between Blood and Urine Arsenic Concentrations and Global Levels of Post-Translational Histone Modifications in Bangladeshi Men and Women. *Environ. Health Perspect* 2016, 124 (8), 1234–1240. 10.1289/ehp.1510412. [PubMed: 26967670]
- (26). Bhowmick S; Halder D; Nriagu J; Guha Mazumder DN; Roman-Ross G; Chatterjee D; Iglesias M Speciation of Arsenic in Saliva Samples from a Population of West Bengal, India. *Environ. Sci. Technol* 2014, 48 (12), 6973–6980. 10.1021/es4056142. [PubMed: 24857783]
- (27). Sethupathy P The Promise and Challenge of Therapeutic MicroRNA Silencing in Diabetes and Metabolic Diseases. *Curr. Diab. Rep* 2016, 16 (6), 52 10.1007/s11892-016-0745-3. [PubMed: 27112956]
- (28). Bartel DP Metazoan MicroRNAs. *Cell* 2018, 173 (1), 20–51. 10.1016/j.cell.2018.03.006. [PubMed: 29570994]
- (29). Iorio MV; Croce CM MicroRNA Dysregulation in Cancer: Diagnostics, Monitoring and Therapeutics. A Comprehensive Review. *EMBO Mol. Med* 2012, 4 (3), 143–159. 10.1002/emmm.201100209. [PubMed: 22351564]
- (30). Bátkai S; Thum T MicroRNAs in Hypertension: Mechanisms and Therapeutic Targets. *Curr. Hypertens. Rep* 2012, 14 (1), 79–87. 10.1007/s11906-011-0235-6. [PubMed: 22052337]
- (31). Vickers KC; Remaley AT MicroRNAs in Atherosclerosis and Lipoprotein Metabolism. *Curr. Opin. Endocrinol. Diabetes. Obes* 2010, 17 (2), 150–155. 10.1097/MED.0b013e32833727a1. [PubMed: 20150807]
- (32). Trajkovski M; Hausser J; Soutschek J; Bhat B; Akin A; Zavolan M; Heim MH; Stoffel M MicroRNAs 103 and 107 Regulate Insulin Sensitivity. *Nature* 2011, 474 (7353), 649–653. 10.1038/nature10112. [PubMed: 21654750]
- (33). He Y; Ding Y; Liang B; Lin J; Kim T-K; Yu H; Hang H; Wang K A Systematic Study of Dysregulated MicroRNA in Type 2 Diabetes Mellitus. *Int. J. Mol. Sci* 2017, 18 (3). 10.3390/ijms18030456.
- (34). Hung Y-H; Sethupathy P Important Considerations for Studies of Circulating MicroRNAs in Clinical Samples. *EBioMedicine* 2017, 24, 22–23. 10.1016/j.ebiom.2017.09.030. [PubMed: 28988980]
- (35). Ludwig N; Leidinger P; Becker K; Backes C; Fehlmann T; Pallasch C; Rheinheimer S; Meder B; Stähler C; Meese E; et al. Distribution of MiRNA Expression across Human Tissues. *Nucleic Acids Res.* 2016, 44 (8), 3865–3877. 10.1093/nar/gkw116. [PubMed: 26921406]

- (36). De Guire V; Robitaille R; Tétreault N; Guérin R; Ménard C; Bambace N; Sapienza P Circulating MiRNAs as Sensitive and Specific Biomarkers for the Diagnosis and Monitoring of Human Diseases: Promises and Challenges. *Clin. Biochem* 2013, 46 (10–11), 846–860. 10.1016/J.CLINBIOCHEM.2013.03.015. [PubMed: 23562576]
- (37). Rager JE; Bailey KA; Smeester L; Miller SK; Parker JS; Laine JE; Drobná Z; Currier J; Douillet C; Olshan AF; et al. Prenatal Arsenic Exposure and the Epigenome: Altered MicroRNAs Associated with Innate and Adaptive Immune Signaling in Newborn Cord Blood. *Environ. Mol. Mutagen* 2014, 55 (3), 196–208. 10.1002/em.21842. [PubMed: 24327377]
- (38). Hayes J; Peruzzi PP; Lawler S; Ebert MS; Sharp PA; Choudhury Y; al., et; Stahlhut C; Slack FJ; Lu J; et al. MicroRNAs in Cancer: Biomarkers, Functions and Therapy. *Trends Mol. Med* 2014, 20 (8), 460–469. 10.1016/j.molmed.2014.06.005. [PubMed: 25027972]
- (39). Kurtz CL; Fannin EE; Toth CL; Pearson DS; Vickers KC; Sethupathy P Inhibition of MiR-29 Has a Significant Lipid-Lowering Benefit through Suppression of Lipogenic Programs in Liver. *Sci. reports* 12911 2015, 5 SRC-G.
- (40). Ying W; Riopel M; Bandyopadhyay G; Dong Y; Birmingham A; Seo JB; Ofrecio JM; Wollam J; Hernandez-Carretero A; Fu W; et al. Adipose Tissue Macrophage-Derived Exosomal MiRNAs Can Modulate In Vivo and In Vitro Insulin Sensitivity. *Cell* 2017, 171 (2), 372–384.e12. 10.1016/j.cell.2017.08.035.
- (41). Yao Z-Y; Chen W-B; Shao S-S; Ma S-Z; Yang C-B; Li M-Z; Zhao J-J; Gao L Role of Exosome-Associated MicroRNA in Diagnostic and Therapeutic Applications to Metabolic Disorders. *J. Zhejiang Univ. Sci. B* 2018, 19 (3), 183–198. 10.1631/jzus.B1600490. [PubMed: 29504312]
- (42). Sollome J; Martin E; Sethupathy P; Fry RC Environmental Contaminants and MicroRNA Regulation: Transcription Factors as Regulators of Toxicant-Altered MicroRNA Expression. *Toxicol. Appl. Pharmacol* 2016 10.1016/j.taap.2016.06.009.
- (43). Schembri F; Sridhar S; Perdomo C; Gustafson AM; Zhang X; Ergun A; Lu J; Liu G; Zhang X; Bowers J; et al. MicroRNAs as Modulators of Smoking-Induced Gene Expression Changes in Human Airway Epithelium. *Proc. Natl. Acad. Sci. U. S. A* 2009, 106 (7), 2319–2324. 10.1073/pnas.0806383106. [PubMed: 19168627]
- (44). Vrijens K; Bollati V; Nawrot TS MicroRNAs as Potential Signatures of Environmental Exposure or Effect: A Systematic Review. *Environ. Health Perspect* 2015 10.1289/ehp.1408459.
- (45). Matoušek T; Currier JM; Trojánková N; Saunders RJ; Ishida MC; González-Horta C; Musil S; Mester Z; Stýblo M; Dina J Selective Hydride Generation- Cryotrapping- ICP-MS for Arsenic Speciation Analysis at Picogram Levels: Analysis of River and Sea Water Reference Materials and Human Bladder Epithelial Cells. *J. Anal. At. Spectrom* 2013, 28 (9), 1456–1465. 10.1039/C3JA50021G. [PubMed: 24014931]
- (46). Grau-Pérez M; Kuo C-C; Spratlen M; Thayer KA; Mendez MA; Hamman RF; Dabelea D; Adgate JL; Knowler WC; Bell RA; et al. The Association of Arsenic Exposure and Metabolism With Type 1 and Type 2 Diabetes in Youth: The SEARCH Case-Control Study. *Diabetes Care* 2017, 40 (1), 46–53. 10.2337/dc16-0810. [PubMed: 27810988]
- (47). Keith BP; Barrow JB; Toyonaga T; Kazgan N; O'Connor MH; Shah ND; Schaner MS; Wolber EA; Trad OK; Gipson GR; et al. Colonic Epithelial MiR-31 Associates with the Development of Crohn's Phenotypes. *JCI Insight* 2018, 3 (19). 10.1172/jci.insight.122788.
- (48). Kanke M; Baran-Gale J; Villanueva J; Sethupathy P MiRquant 2.0: An Expanded Tool for Accurate Annotation and Quantification of MicroRNAs and Their IsomiRs from Small RNA-Sequencing Data. *J. Integr. Bioinform* 2016, 13 (5), 307 10.2390/biecoll-jib-2016-307. [PubMed: 28187421]
- (49). Permeth-Wey J; Chen D-T; Fulp WJ; Yoder SJ; Zhang Y; Georgeades C; Husain K; Centeno BA; Magliocco AM; Coppola D; et al. Plasma MicroRNAs as Novel Biomarkers for Patients with Intraductal Papillary Mucinous Neoplasms of the Pancreas. *Cancer Prev. Res* 2015, 8 (9), 826–834. 10.1158/1940-6207.CAPR-15-0094.
- (50). Rizzo R; Ragusa M; Barbagallo C; Sammito M; Gulisano M; Cali PV; Pappalardo C; Barchitta M; Granata M; Condorelli AG; et al. Circulating MiRNAs Profiles in Tourette Syndrome: Molecular Data and Clinical Implications. *Mol. Brain* 2015, 8 (1), 44 10.1186/s13041-015-0133-y. [PubMed: 26205656]

- (51). Narla V; Bhakta N; Freedman JE; Tanriverdi K; Maka K; Deeks SG; Ganz P; Hsue P Unique Circulating MicroRNA Profiles in HIV Infection. *JAIDS J. Acquir. Immune Defic. Syndr* 2018, 1 10.1097/QAI.0000000000001851.
- (52). Tong D GT What Is Normal? Next Generation Sequencing-Driven Analysis of the Human Circulating MiRNAome. *BMC Mol. Biol* 2016, 17 (1), 4. [PubMed: 26860190]
- (53). Chen C-J; Wang S-L; Chiou J-M; Tseng C-H; Chiou H-Y; Hsueh Y-M; Chen S-Y; Wu M-M; Lai M-S Arsenic and Diabetes and Hypertension in Human Populations: A Review. *Toxicol. Appl. Pharmacol* 2007, 222 (3), 298–304. 10.1016/j.taap.2006.12.032. [PubMed: 17307211]
- (54). Navas-Acien A; Silbergeld EK; Streeter RA; Clark JM; Burke TA; Guallar E Arsenic Exposure and Type 2 Diabetes: A Systematic Review of the Experimental and Epidemiological Evidence. *Environ. Health Perspect* 2006, 114 (5), 641–648. [PubMed: 16675414]
- (55). Prakash C; Soni M; Kumar V Mitochondrial Oxidative Stress and Dysfunction in Arsenic Neurotoxicity: A Review. *J. Appl. Toxicol* 2016, 36 (2), 179–188. 10.1002/jat.3256. [PubMed: 26510484]
- (56). Sinha D; Biswas J; Bishayee A Nrf2-Mediated Redox Signaling in Arsenic Carcinogenesis: A Review. *Arch. Toxicol* 2013, 87 (2), 383–396. 10.1007/s00204-012-0920-5. [PubMed: 22914984]
- (57). Muenyi CS; Ljungman M; States JC Arsenic Disruption of DNA Damage Responses-Potential Role in Carcinogenesis and Chemotherapy. *Biomolecules* 2015, 5 (4), 2184–2193. 10.3390/biom5042184. [PubMed: 26404387]
- (58). Kessel M; Liu SX; Xu A; Santella R; Hei TK Arsenic Induces Oxidative DNA Damage in Mammalian Cells. *Mol. Cell. Biochem* 234–235 (1–2), 301–308.
- (59). Nesnow S; Roop BC; Lambert G; Kadiiska M; Mason RP; Cullen WR; Mass MJ DNA Damage Induced by Methylated Trivalent Arsenicals Is Mediated by Reactive Oxygen Species. *Chem. Res. Toxicol* 2002, 15 (12), 1627–1634. [PubMed: 12482246]
- (60). Hou L; Wang D; Baccarelli A Environmental Chemicals and MicroRNAs. *Mutat. Res. - Fundam. Mol. Mech. Mutagen* 2011, 714 (1–2), 105–112. 10.1016/j.mrfmmm.2011.05.004.
- (61). Sturchio E; Colombo T; Boccia P; Carucci N; Meconi C; Minoia C; Macino G Arsenic Exposure Triggers a Shift in MicroRNA Expression. *Sci. Total Environ* 2014, 472 (528), 672–680. 10.1016/j.scitotenv.2013.11.092. [PubMed: 24317173]
- (62). Sanders AP; Messier KP; Shehee M; Rudo K; Serre ML; Fry RC Arsenic in North Carolina: Public Health Implications. *Environ. Int* 2012, 38 (1), 10–16. 10.1016/j.envint.2011.08.005. [PubMed: 21982028]
- (63). Bommarito P; Beck R; Douillet C; Del Razo LM; Garcia-Vargas GG; Valenzuela Olga L. Castellanos EH; Sánchez-Peña, Sethupathy Luz C., Praveen Stýblo M; Fry RC. Validation of Plasma Arsenicals as Biomarkers of Chronic Exposure to Inorganic Arsenic. submitted 2018.
- (64). Thomas DJ; Li J; Waters SB; Xing W; Adair BM; Drobna Z; Devesa V; Styblo M Arsenic (+3 Oxidation State) Methyltransferase and the Methylation of Arsenicals. *Exp. Biol. Med.* (Maywood) 2007, 232 (1), 3–13. [PubMed: 17202581]
- (65). Vahter M; Concha G Role of Metabolism in Arsenic Toxicity. *Pharmacol. Toxicol* 2001, 89 (1), 1–5.
- (66). Styblo M; Del Razo LM; Vega L; Germolec DR; LeCluyse EL; Hamilton GA; Reed W; Wang C; Cullen WR; Thomas DJ Comparative Toxicity of Trivalent and Pentavalent Inorganic and Methylated Arsenicals in Rat and Human Cells. *Arch. Toxicol* 2000, 74 (6), 289–299. [PubMed: 11005674]
- (67). Douillet C; Currier J; Saunders J; Bodnar WM; Matousek T; Stýblo M. Methylated Trivalent Arsenicals Are Potent Inhibitors of Glucose Stimulated Insulin Secretion by Murine Pancreatic Islets. *Toxicol. Appl. Pharmacol* 2013, 267 (1), 11–15. DOI: 10.1016/j.taap.2012.12.007 [PubMed: 23261974]
- (68). Paul DS; Harmon AW; Devesa V; Thomas DJ; Stýblo M. Molecular Mechanisms of the Diabetogenic Effects of Arsenic Inhibition of Insulin Signaling by Arsenite and Methylarsonous Acid. *Environ. Health Perspect* 2007, 115 (5), 734–742. 10.1289/ehp.9867. [PubMed: 17520061]

- (69). Zhang C; Fennel EMJ; Douillet C; Stýblo M Exposures to Arsenite and Methylarsonite Produce Insulin Resistance and Impair Insulin-Dependent Glycogen Metabolism in Hepatocytes. *Arch. Toxicol* 2017, 91 (12), 3811–3821. 10.1007/s00204-017-2076-9. [PubMed: 28952001]
- (70). Kuo C-C; Moon KA; Wang S-L; Silbergeld E; Navas-Acien A The Association of Arsenic Metabolism with Cancer, Cardiovascular Disease, and Diabetes: A Systematic Review of the Epidemiological Evidence. *Environ. Health Perspect* 2017, 125 (8), 087001. 10.1289/EHP577.
- (71). Melak D; Ferreccio C; Kalman D; Parra R; Acevedo J; Pérez L; Cortés S; Smith AH; Yuan Y; Liaw J; et al. Arsenic Methylation and Lung and Bladder Cancer in a Case-Control Study in Northern Chile. *Toxicol. Appl. Pharmacol* 2014, 274 (2), 225–231. 10.1016/j.taap.2013.11.014. [PubMed: 24296302]
- (72). Chen Y; Wu F; Liu M; Parvez F; Slavkovich V; Eunus M; Ahmed A; Argos M; Islam T; Rakibuz-Zaman M; et al. A Prospective Study of Arsenic Exposure, Arsenic Methylation Capacity, and Risk of Cardiovascular Disease in Bangladesh. *Environ. Health Perspect* 2013, 121 (7), 832–838. 10.1289/ehp.1205797. [PubMed: 23665672]
- (73). Kuo C-C; Howard BV ; Umans JG; Gribble MO; Best LG; Francesconi KA; Goessler W; Lee E; Guallar E; Navas-Acien A Arsenic Exposure, Arsenic Metabolism, and Incident Diabetes in the Strong Heart Study. *Diabetes Care* 2015, 38 (4), 620–627. 10.2337/dc14-1641. [PubMed: 25583752]
- (74). Ortega FJ; Mercader JM; Moreno-Navarrete JM; Rovira O; Guerra E; Esteve E; Xifra G; Martínez C; Ricart W; Rieusset J; et al. Profiling of Circulating MicroRNAs Reveals Common MicroRNAs Linked to Type 2 Diabetes That Change with Insulin Sensitization. *Diabetes Care* 2014, 37 (5), 1375–1383. 10.2337/dc13-1847. [PubMed: 24478399]
- (75). Yang W; Wang J; Chen Z; Chen J; Meng Y; Chen L; Chang Y; Geng B; Sun L; Dou L; et al. NFE2 Induces MiR-423–5p to Promote Gluconeogenesis and Hyperglycemia by Repressing the Hepatic FAM3A-ATP-Akt Pathway. *Diabetes* 2017, 66 (7), 1819–1832. 10.2337/db16-1172. [PubMed: 28411267]
- (76). Nabiałek E; Wa ha W; Kula D; Jadczyk T; Krajewska M; Kowalówka A; Dworowy S; Hrycek E; Włodarczyk W; Parma Z; et al. Circulating MicroRNAs (MiR-423–5p, MiR-208a and MiR-1) in Acute Myocardial Infarction and Stable Coronary Heart Disease. *Minerva Cardioangiol.* 2013, 61 (6), 627–637. [PubMed: 24253456]
- (77). Collares CVA; Evangelista AF; Xavier DJ; Rassi DM; Arns T; Foss-Freitas MC; Foss MC; Puthier D; Sakamoto-Hojo ET; Passos GA; et al. Identifying Common and Specific MicroRNAs Expressed in Peripheral Blood Mononuclear Cell of Type 1, Type 2, and Gestational Diabetes Mellitus Patients. *BMC Res. Notes* 2013, 6, 491 10.1186/1756-0500-6-491. [PubMed: 24279768]
- (78). Jiao M; You H-Z; Yang X-Y; Yuan H; Li Y-L; Liu W-X; Jin M; Du J Circulating MicroRNA Signature for the Diagnosis of Childhood Dilated Cardiomyopathy. *Sci. Rep* 2018, 8 (1), 724 10.1038/s41598-017-19138-4. [PubMed: 29335596]
- (79). Nair N; Kumar S; Gongora E; Gupta S Circulating MiRNA as Novel Markers for Diastolic Dysfunction. *Mol. Cell. Biochem* 2013, 376 (1–2), 33–40. 10.1007/s11010-012-1546-x. [PubMed: 23247724]
- (80). Deli D; Eisele C; Schmid R; Baum P; Wiech F; Gerl M; Zimdahl H; Pullen SS; Urquhart R Urinary Exosomal MiRNA Signature in Type II Diabetic Nephropathy Patients. *PLoS One* 2016, 11 (3), e0150154. 10.1371/journal.pone.0150154.

Figure 1: Correlations between log-transformed concentrations of As in drinking water ($\mu\text{g/L}$) and log-transformed plasma concentrations of iAs (A); MAs (B); DMAs (C); and TAs (D) (ng/mL).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2:
The top 20 most abundant circulating miRNAs.

Figure 3: MiRNAs associated with plasma arsenicals. The Venn diagrams show 40 shared miRNAs significantly ($P < 0.05$) associated MAs, DMAs, and TAs (A), and 2 miRNAs shared between iAs, MAs, and DMAs (B) before FDR adjustments.

Figure 4: MiRNAs associated with plasma arsenicals after FDR adjustments. Significant associations ($q < 0.15$) were found only between miRNAs and MAs.

Table 1:

Basic characteristics of the study participants.

	Mean (IQR ^I)
Population	82
Sex	
Male	22
Female	60
Smoking	
Non-Smoker	78
Smokers	4
Alcohol consumption	
No	66
Yes	16
Age (years)	34 (30)
BMI (Kg/m ²)	27.09 (7.77)

^IIQR: The interquartile range is defined as the difference between the upper quartile (Q4; the highest 25%) and the lower quartile (Q1; the lowest 25%) of a data set.

Table 2:Concentrations of As in drinking water and of As species in fasting plasma¹

Arsenic Measure	N	Median (IQR)	Range
Concentration of As in water (µg/L)	82	15 (38.05)	3.1 – 215.2
Concentrations of As species in plasma (ng As/mL)	82		
iAs		0.188 (0.115)	0.019 – 5.071
MAs		0.221 (0.267)	0.047 – 2.844
DMAs		0.347 (0.451)	0.083 – 3.155
TAs		0.749 (0.845)	0.221 – 9.670
Distribution of As species in plasma (% of TAs) ²	82		
iAs		25.20% (22.75)	3.9% – 52.5%
MAs		28.23% (9.3683)	15.96% – 46.9%
DMAs		45.07% (14.9)	18.135% – 69.8%

¹Concentrations of As species in all water and plasma samples were above the limits of detection²TAs, total As is defined as sum of iAs+MAs+DMAs

Table 3–

MiRNAs associated with plasma MAs.

MiRNA	Partial Correlation Coefficient ^I	P Value	q Value
hsa-miR-423-5p	0.356473	0.00231129	0.139448
hsa-miR-142-5p -2	-0.39488	0.00138898	0.125703
hsa-miR-423-5p +1	0.384065	0.00220734	0.139448
hsa-miR-320c-1	0.363683	0.00116779	0.125703
hsa-miR-320c-2	0.366302	0.00098734	0.125703
hsa-miR-454-5p	-0.39685	0.00099197	0.125703

^IPartial correlation coefficients were calculated with a linear regression model controlling for age, sex, BMI, smoking status, and alcohol consumption.