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Sex-specific patterns and deregulation of endocrine pathways in the gene expression profiles of Bangladeshi adults exposed to arsenic contaminated drinking water

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ABSTRACT

Arsenic contamination of drinking water occurs globally and is associated with numerous diseases including skin, lung and bladder cancers, and cardiovascular disease. Recent research indicates that arsenic may be an endocrine disruptor. This study was conducted to evaluate the nature of gene expression changes among males and females exposed to arsenic contaminated water in Bangladesh at high and low doses. Twenty-nine (55% male) Bangladeshi adults with water arsenic exposure ranging from 50 to 1000 μg/L were selected from the Folic Acid Creatinine Trial. RNA was extracted from peripheral blood mononuclear cells for gene expression profiling using Affymetrix 1.0 ST arrays. Differentially expressed genes were assessed between high and low exposure groups for males and females separately and findings were validated using quantitative real-time PCR. There were 534 and 645 differentially expressed genes ($p < 0.05$) in the peripheral blood mononuclear cells of males and females, respectively, when high and low water arsenic exposure groups were compared. Only 43 genes overlapped between the two sexes, with 29 changing in opposite directions. Despite the difference in gene sets both males and females exhibited common biological changes including deregulation of 17β-hydroxysteroid dehydrogenase enzymes, deregulation of genes downstream of Sp1 (specificity protein 1) transcription factor, and prediction of estrogen receptor alpha as a key hub in cardiovascular networks. Arsenic-exposed adults exhibit sex-specific gene expression profiles that implicate involvement of the endocrine system. Due to arsenic's possible role as an endocrine disruptor, exposure thresholds for arsenic may require different parameters for males and females.

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Introduction

Inorganic arsenic (iAs), a known human carcinogen, naturally contaminates drinking water and currently affects approximately 150 million people in at least 70 countries throughout the world (IARC, 2004; Ravenscroft et al., 2009). Exposure to As is associated with skin, lung, bladder, liver and prostate cancers (Benbrahim-Tallaa and Waalkes, 2008; Rahman et al., 2009), cardiovascular disease (Chen et al., 2013; Tseng et al., 2003), diabetes (Pan et al., 2013), and hypertension (Chen et al., 1995). The mechanisms that underlie arsenic's diverse pathogenesis remain unclear and are thought to include oxidative stress, endocrine disruption, alteration of cell signaling and proliferation processes, and impairment of DNA damage responses (Andrew et al., 2003; Davey et al., 2007; Flora, 2011; Schuhmacher-Wolz et al., 2009). In addition, there is a growing body of evidence to indicate that As acts through epigenetic mechanisms as part of its pathogenesis by altering the abundance of histone modifications and the level of DNA methylation (Reichard and Puga, 2010; Ren et al., 2011).

The evidence to suggest that As acts as an endocrine disruptor includes in vitro activation of steroid hormone receptors (Bodwell et al., 2006; Davey et al., 2007), animal models with altered fertility (Chang et al., 2007; Jana et al., 2006) and sex-specific disease distributions (Waalkes et al., 2003), as well as human studies indicating altered fertility (Hsieh et al., 2008; Xu et al., 2012), sex-specific disease distributions (Aballay et al., 2012), and sex-linked changes in methylation profiles (Pilsner et al., 2012). Previous research from our lab supports this

Abbreviations: AR, androgen receptor; As, arsenic; CVD, cardiovascular disease; ER, estrogen receptor; FACT, Folic Acid Creatinine Trial; GR, glucocorticoid receptor; HEALS, Health Effects of Arsenic Longitudinal Study; HSD17B, 17B-Hydroxysteroid dehydrogenase; HSD, hydroxysteroid dehydrogenase; SCAN, single channel array normalization; Sp1, specificity protein 1; SR, steroid receptors; uAs, urinary As; wAs, water As

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trend with the observed sex-specific changes in the abundance of histone modifications in Bangladeshi adults exposed to As contaminated drinking water (Chervona et al., 2012). Microarray studies that have assessed gene expression changes in response to As contaminated water have evaluated changes with males and females pooled together (Andrew et al., 2008; Lu et al., 2001; Wu et al., 2003) or with only females (Wu et al., 2003). However, given the potential endocrine activity of As, in particular its possible role as a xenoestrogen (Jana et al., 2006; Waalkes et al., 2004), division of sexes in assessing its impact may be necessary to understand its pathogenesis. Indeed a recent study conducted among Flemish adults exposed to an array of toxins, including xenoestrogens, exhibited sex-specific transcriptomic responses (De Coster et al., 2013) that were revealed when sexes were evaluated separately.

Here we evaluate the gene expression profiles of RNA from the peripheral blood mononuclear cells (PBMC) of Bangladeshi adults (n = 29) chronically exposed to As contaminated drinking water. Subjects with high water As exposure are compared to those with low water As exposure among males and females separately. To evaluate the sex-specific profiles we compare the areas of biological relevance for each sex and evaluate to what extent the same genes are found among biologically relevant areas that are common to males and females.

Materials and methods

Study site and subject recruitment. The Health Effects of Arsenic Longitudinal Study (HEALS) cohort is part of Columbia University's Superfund Research Program and currently includes approximately 30,000 participants (Ahsan et al., 2006). The present study utilizes samples from a subset of 600 HEALS participants who were recruited for enrollment into the Folic Acid and Creatine Trial (FACT), a clinical trial that aimed to lower blood As concentrations with nutritional supplementation and therefore required that participants be drinking from wells with water As concentrations greater than or equal to 50 μg/L. Fieldwork was completed in June 2011. Study participants were given a water filtration system for removal of As at the time of enrollment. Participants with renal or gastrointestinal diseases, those taking nutritional supplements, or who were pregnant and/or planning to become pregnant were not included in the study. This study evaluates the gene expression patterns of 29 study participants from the FACT clinical trial. Our study population is comprised of adults ranging in age from 26 to 53. Here the effects of relatively low exposure (50–200 μg/L) to water arsenic are compared to high exposure (250–1000 μg/L) for males (n = 16) and females (n = 13) separately. Among the males there were 9 males with low exposure and 7 with high exposure. Among the females there were 6 females with low exposure and 7 with high exposure. Please see Table 1 for other information regarding the demographic features of the groups.

Sample collection and handling. Blood samples were obtained by venipuncture, and collected in EDTA-containing vacutainer tubes, which were then placed in IsoRack/IsoPack cool packs (Brinkmann Instruments). Samples were transported in hand-carried coolers to the local laboratory at the field clinic in Araihazar, within 4 h of collection. Samples were centrifuged for 10 min at 3000 × *g* at 4 °C, and the plasma fraction was stored at −80 °C. PBS was added to the remaining cells followed by a ficoll-hypaque gradient extraction of PBMCs using standard techniques. PBMCs were stored at −80 °C. Urine samples were collected with 50-mL acid-washed polypropylene tubes. Within 4 h samples were transported from portable coolers to freezers (−20 °C) in the Araihazar laboratory, and were then hand carried on dry ice to New York.

Water and urinary As. Water sample collection, sample handling, and analysis for this study were performed as previously described (Chervona et al., 2012). The water As samples were analyzed at Columbia University's Lamont Doherty Earth Observatory by inductively coupled mass spectrometry (ICP–MS), with a detection limit of 0.1 μg/L. Total urinary As (uAs) concentrations were measured at Columbia University Trace Metals Core Lab by GFAA spectrometry using an AAnalyst 600 graphite furnace system (PerkinElmer), as described (Nixon et al., 1991). This laboratory is part of a quality control program run by the Institut de Sante Publique du Quebec, Canada. Intraclass correlation coefficients (ICCs) were 0.99 between the Columbia laboratory's values and samples calibrated at the Quebec laboratory. To correct for hydration status, urinary creatinine was analyzed using a method based on the Jaffe reaction.

RNA isolation, amplification, and hybridization. Total RNA was extracted from each sample according to the TRIzol (Invitrogen) manufacturer's protocol, and purified using RNeasy Plus Micro Kit (Qiagen). Sense strand cDNA probes were synthesized (amplified) using an Ambion Whole Transcript Expression Kit. Amplified single stranded cDNA was fragmented and labeled using a GeneChip WT Terminal Labeling Kit (Affymetrix). The fragmented and labeled DNA underwent hybridization with Affymetrix GeneChip Human Gene 1.0 ST Array that contains 28,869 well-annotated genes. Samples were processed in two batches

Table 1
General characteristics for the total study sample by sex and water As group[a].

	Total sample (n = 29)	Males, low water As[b] (n = 9)	Males, high water As[c] (n = 7)	Females, low water As[d] (n = 6)	Females, high water As[e] (n = 7)
Age	40.0 ± 7.2	42.6 ± 6.4	41.7 ± 6.8	36.7 ± 9.6	37.7 ± 5.6
BMI, kg/m^2	20.4 ± 2.9	19.7 ± 2.1	18.2 ± 1.1	23.8 ± 2.1	20.6 ± 3.0
Education, years	2.9 ± 3.7	2.8 ± 3.4	0	5.0 ± 4.5	4.0 ± 3.8
Water As μg/L	246.4 ± 207.4	103.0 ± 33.7	354.5 ± 136.2	116.7 ± 61.9	433.9 ± 274.2
Urinary As, μg/L	264.1 ± 349.3	116.8 ± 76.4	456.3 ± 597.3	113.3 ± 99.7	390.6 ± 281.7
Urinary creatinine (mg/dL)	75.0 ± 41.5	75.3 ± 40.9	58.0 ± 38.8	74.5 ± 54.7	91.9 ± 33.9
Urinary As/mg creatinine	345.8 ± 324.9	165.5 ± 74.9	650.2 ± 450.7	178.9 ± 186.7	416.3 ± 250.1
Blood As (μg/L)	11.4 ± 10.3	6.8 ± 3.1	20.7 ± 15.3	7.1 ± 7.2	11.7 ± 7.3
Male, %	55.2	–	–	–	–
Land ownership, %	37.9	44.4	42.9	33.3	28.6
Television ownership, %	24.1	11.1	14.3	33.3	42.9
Current cigarette smoking, %	44.8	77.8	85.7	0	0
Current betel nut use, %	34.5	55.6	42.9	16.7	14.3

[a] Mean ± SD unless otherwise noted.
[b] Water As 50 to 150 μg/L.
[c] Water As 232 to 500 μg/L.
[d] Water As 50 to 200 μg/L.
[e] Water As 250 to 1000 μg/L.

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