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Arsenic exposure, telomere length, and expression of telomere-related genes among Bangladeshi individuals

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Abstract

Background—Inorganic arsenic is a carcinogen whose mode of action may involve telomere dysfunction. Recent epidemiological studies suggest that chronic arsenic exposure is associated with longer telomeres and altered expression of telomere-related genes in peripheral blood. In this study, we evaluated the association of urinary arsenic concentration with expression of telomere-related genes and telomere length in Bangladeshi individuals with a wide range of arsenic exposure through naturally contaminated drinking water.

Methods—We used linear regression models to estimate associations between urinary arsenic and array-based expression measures for 69 telomere related genes using mononuclear cell RNA samples from 1,799 individuals. Association between arsenic exposure and a qPCR-based telomere length was assessed among 167 individuals.

Results—Urinary arsenic was possitively associated with expression of WRN, and negatively associated with TERF2, DKC1, TERF2IP and OBFC1 (all $P < 0.00035$, Bonferroni correction threshold). We detected interaction between urinary arsenic and arsenic metabolism efficiency in relation to expression of WRN (P for interaction = 0.00008). In addition, we observed that very

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high arsenic exposure was associated with longer telomeres compared to very low exposure (P=0.02).

Discussion—Our findings suggest that arsenic's carcinogenic mode of action may involve alteration of telomere maintenance and/or telomere damage. This study extends our knowledge regarding the effect of arsenic on telomere length and expression of telomere-related genes.

1. Introduction

The telomere is a tandem repeat sequence $(TTAGGG)_n$ at the end of human chromosomes that binds a protein complex to protect chromosome ends from erosion and chromosome fusion events. In differentiated cells, the telomere sequences shorten with each cell division due to the "end replication problem" (Olovnikov, 1973), eventually triggering senscence. In stem cells, telomere length is maintained by telomerase, but length grandually decreases over the lifecourse (Finkel et al., 2007; McEachern et al., 2000). Telomerase activity is low or absent in most differentiated cells, resulting in a finite capacity for replication. Short telomere length in peripheral blood leukocytes is often considered a general biomarker of aging (Butler et al., 2004), but both shorter and longer telomeres have been associated with risk of a number of chronic diseases (Cawthon et al., 2003; Fitzpatrick et al., 2007; Sanders et al., 2012) and cancers (Han et al., 2009; Nan et al., 2011a; Shen et al., 2011; Stewart and Weinberg, 2000; Zimmermann and Martens, 2008).

As a potential biomarker of cancer risk, telomere length may be influenced by cancer-causing agents, such as arsenic. Exposure to inorganic arsenic through contaminated drinking water affects >100 million people worldwide (Ravenscroft et al., 2009), and chronic exposure to high levels (e.g., >300 μg/L in drinking water) shows clear association with increased risk for cancers of the lung (Celik et al., 2008), bladder (Mink et al., 2008), liver (Liu and Waalkes, 2008), skin (Yu et al., 2006), and kidney (Chen et al., 1992; Yuan et al., 2010). The mechanisms by which arsenic contributes to carcinogenesis are not entirely understood. Arsenic is not believed to directly damage DNA (Basu et al., 2001), and a variety of potential modes of action for arsenic toxicity have been proposed (Bhattacharjee et al., 2013; Hughes, 2002; Hughes et al., 2011; Kitchin, 2001; Schoen et al., 2004). These include oxidative stress, reduced DNA repair capacity, and increased cell turnover due to cytotoxicity, all of which could potentially damage telomeres.

Experimental studies suggest that arsenic has dose-dependent effects on telomere length and telomerase. Studies of cell lines (Zhang et al., 2003), animal models (Liu et al., 2003), and human cells (Ferrario et al., 2009) indicate that arsenic reduces telomere length at high concentrations (>1 μM), decreasing cell survival, but may increase telomerase activity and main telomere length at lower concentrations (<1 μM) (increasing survival). Recent epidemiological studies suggest that chronic arsenic exposure is associated with longer telomeres in peripheral blood leukocytes (Chatterjee et al., 2014; Li et al., 2012) and increased expression of telomerase (Mo et al., 2009b), although few studies have addressed these research questions in human populations. In the present study, we estimated the association between arsenic exposure and expression of 69 telomere-related genes in mononuclear cells among 1,799 Bangladeshi individuals. We also estimate the association

between arsenic exposure and telomere length in peripheral blood in separate sample of 167 Bangladeshi individuals.

2. Materials and methods

2.1. Study participants

Participants for this work were drawn from two longitudinal studies in Bangladeshi with similar source populations: the Bangladesh Vitamin E and Selenium Trial (BEST) (Argos et al., 2013) and the Health Effects of Arsenic Longitudinal Study (HEALS) (Ahsan et al., 2006).

BEST is a randomized chemoprevention trial evaluating the long-term effects of vitamin E or selenium supplementation on non-melanoma skin cancer (NMSC) risk and oxidative stress. BEST participants(n=7,000) are adult residents in Araihazar, Matlab, and surrounding areas in Bangladesh with prevalent arsenic-related skin lesions (Argos et al., 2013). For all participants, biological samples were collected at the time of the baseline interview prior to dietary supplementation, including urine samples for arsenic measurement and mononuclear cells for RNA isolation. 1,799 individuals were randomly selected from BEST for genome-wide expression profiling.

HEALS is a large prospective cohort study designed to investigate the health effects of arsenic exposure through drinking water in a population-based sample of adults in Araihazar, Bangladesh. Details of the study design have been published previously (Ahsan et al., 2006). At baseline (2000–2002), study interviewers collected information on demographic and lifestyle characteristics, conducted clinical examinations, and obtained bio-specimens (blood and urine). We measured telomere length among 167 participants selected from the extremes of the arsenic exposure distribution to enhance power to detect an association. High exposure was defined as arsenic concentration >145 μg/L in water and >339 μg/g in urine (measured at baseline). Low exposure was defined as <12 μg/L in water and <107 μg/g in urine.

Data for 1,799 BEST participants were used to estimate associations between arsenic exposure and gene expression levels. Data on 167 HEALS participants were used to estimate the association between arsenic exposure and telomere length.

2.2. Measurements of arsenic in water and urine

In each study, urinary arsenic was measured using graphite furnace atomic absorption spectrometry in a single laboratory (Nixon et al., 1991). Urinary arsenic was measured at the Trace Metals Core Laboratory at Columbia University, which is a member of the quality control program run by Institute de Sante Publique du Quebec and uses their quality control samples to standardize the measurements of urinary arsenic. The laboratory has consistently measured urinary arsenic concentration with correlation >0.97 for blinded quality control samples. Urinary creatinine was measured by a colorimetric diagnostics kit (Sigma, St Louis, MO, USA). Total urinary arsenic concentration was divided by creatinine to obtain creatinine-adjusted total arsenic concentration (μg/g creatinine) (Nermell et al., 2008). Urinary arsenic metabolite data were not available for the majority of our study participants

but have been previously measured in a subset. In the subset we observed a very low proportion of total urinary arsenic to be arsenocholine or arsenobetaine (3%) (Ahsan et al., 2007; Argos et al., 2010), most likely due to the relatively low seafood intake in the study region.

In HEALS, we also measured arsenic concentrations in drinking water at the baseline interview. Arsenic concentrations were analyzed by graphite furnace atomic absorption or by inductively coupled plasma-mass spectrometry when concentrations were below 5 μg/L. More detailed information about methods and QC have been described in our prior paper (van Geen et al., 2003).

2.3. Whole Genome Gene Expression Profiling

Total RNA was extracted from peripheral blood mononuclear cells (PBMC) preserved in buffer RLT, and stored at −86°C using RNeasy Micro Kit (cat# 74004) from Qiagen, Valencia, USA. Concentration and quality of all extracted RNA were checked on Nanodrop 1000. Following Illumina protocols, we convereted RNA to cDNA and then to cRNA which was run on HT12 V4 BeadChip Expression Arrays. The chip contains a total of 47,231 probes covering 31,335 genes.

2.4. Selection of telomere-related genes for expression analysis

Telomere-related genes were selected based on prior literature. We selected genes related to the shelterin complex, genes involved in telomere maintence, as well as genes that are transiently associated with the telomere, including gene involved in DNA repair and helicase genes, (as classfied previously (Mirabello et al., 2010)). The majority of our candidate genes were selected based on three prior studies of telomere-related genes (Mirabello et al., 2012; Mirabello et al., 2010; Nan et al., 2011b) and one study of arsenic exposure, telomere length, and gene expression (Li et al., 2012). We also included all 11 genes reported in recent genomewide association studies of telomere length (Codd et al., 2010; Gu et al., 2011; Levy et al., 2010; Mangino et al., 2012; Mangino et al., 2009; Prescott et al., 2011): ZNF676, CTC1, OBFC1, TERC, DHX35, WDR65, PELI2, KPNA5, SLC44A4, CXCR4 and CELF4 (BRUNOL4). In addition, we included 2 additional genes related to telomere biology (OGG1 and ERCC1) (Lu and Liu, 2010; Vannier et al., 2009) whose expression was reported to be associated with arsenic exposure among individuals in Inner Mongolia, China (Mo et al., 2009a; Mo et al., 2006). In total, we identified 71 genes of interest.

To ensure each probe mapped uniquely to a single gene, we aligned the Illumina probe sequences to all transcriptome sequences contained in both the known Gene mRNA and the known Gene Tx-mRNA tables from the UCSC Genome Browser (version GRCh37/hg19), as recommended by Barbosa-Morais et al. (Barbosa-Morais et al., 2010). After excluding probes that mapped to multiple genes, we identified 31,853 probes were specific to 20,143 genes. Among the 71 selected genes, 143 probes mapping to 69 genes remained for analysis (Supplementary Table S1&S2).

2.5. Assessment of telomere length

Our small (pilot) study of telomere length was conducted in HEALS because HEALS has a wider exposure distribution than BEST, so were better able to select individuals at the extremes of exposure (83 with “very high” exposure and 84 with “very low” exposure). Relative telomere length (TL) was measured for 167 HEALS participants using DNA samples extracted from clot blood using Flexigene DNA kit (Cat # 51204) from Qiagen (5ng/μl) (Pierce et al., 2012). A high-throughput SYBR Green RT-PCR assay was carried out on an Applied Biosystems (ABI) RT-PCR System and software in 96-well format. Using 10 ng of DNA, mean telomere content relative to genomic DNA was measured using two RT-PCR reactions (each in quadruplicate), one amplifying a 78 bp telomereic repeat unit (T) and the other amplifying a single-copy housekeeping gene (S), 36B4U, as described by Cawthon, et al (Cawthon, 2002). The fluorescent signal from the T reaction is proportional to the number of telomere repeat units in the genome available for primer binding. The S signal is used to account for between-sample variation in abundance of template DNA. Reaction efficiencies (the steepness of fluorescence increase, calculated using LinRegPCR software) and the crossing-point deviation of an unknown sample versus a control was used to calculate T/S ratios corrected for betweenindividual and between-gene differences in efficiency (Pfaffl, 2001). This method produces a highly accurate and reproducible T/S ratio, which is proportional to the relative telomere length of an individual. All samples were randomly distributed across plates. For quadruplicate measures with coefficients of variation (CV) of <5% for cycle threshold, we excluded an outlying value to ensure CVs<5%. Laboratory technicians were trained to adhere to the MIQE guidelines.

2.6. Statistical analyses

We used linear regression to estimate the association between creatinine-adjusted total urinary arsenic and gene expression traits (i.e. probes, log10 transformed), adjusting for gender, age (continuous), experimental batch, smoking status (current, former, and never) and BMI (< 18.5, < 25 and ≥ 25). Because of some extreme exposure values, analyses were performed both with and without log-transformation of urinary arsenic. Arsenic metabolism-related variants in the *AS3MT* region, identified in our recent GWAS, were used to represent arsenic metabolism efficiency (coded as 0 to 4 risk alleles for rs9527 (A) and rs11191527 (G)) (Pierce et al., 2012). We estimated the association between arsenic metabolism efficiency and gene expression. In addition, we assessed the interaction between urinary arsenic exposure (split by median=158.02 ug/g) and SNP-based arsenic metabolism efficiency (0,1,2,3,4) using linear regression model adjusting for sex, age, experimental batch, smoking status, and BMI. For those probes with p for interaction surpassing the multiple correction p value, we examined the association between arsenic and expression stratified by SNP-based arsenic metabolism efficiency (Pierce et al., 2012). Because gene expression of some telomere-related genes may differ by arsenic exposure or sex, we conducted analyses stratified by low/high arsenic exposure (split by median) and by sex. A Bonferroni correction was used to account for multiple testing (143 tests, p threshold = 0.05/143 = 0.00035) as well as a false discovery rate (FDR) of 0.05.

In HEALS, the association between arsenic exposure status (high vs. low) and telomere length was estimated using linear regression adjusting for gender, age (four groups), BMI (<

18.5, < 25 and ≥ 25), and smoking status (smoker and non-smoker). The association between SNP-based arsenic metabolism efficiency and telomere length was also estimated.

All statistical analyses were performed using SAS Version 9.3 (SAS Institute, Inc., Cary, NC, USA). Q values according for FDR were calculated by Q-VALUE software package (Storey, 2003).

2.7. Effect of arsenic on telomere-related genes in human cell lines

We searched the Gene Expression Omnibus (GEO) database to determine if our differentially-expressed genes were also associated with arsenic exposure in human cell lines (http://www.ncbi.nlm.nih.gov/geoprofiles). Using the search terms of "human" and "arsenic" and a specific gene ID (such as "TERF2" or "WRN" etc), we identified one study of human cells (hepatoma HepG2) with data on the most genes analyzed in this work: "Heavy metals effect on liver cell line" (Kawata et al., 2007). The relationship between arsenic and gene expression level was tested using the non-parametric Exact Wilcoxon Two-Sample Test. Because this is a test of the hypothesis that the association is in the same direction as in our gene expression analyses, we applied one-sided tests to assess significance.

2.8. Protocol approvals and participant consent

The study protocol was approved by the Institutional Review Boards of The University of Chicago, Columbia University, and the Bangladesh Medical Research Council and all study participants provided informed consent.

3. Results

Participant characteristics are shown in Table 1. Urinary arsenic concentrations ranged from 1.3 to 4,004 μg/g (median = 158.02 μg/g) in BEST and from 8 to 8,556 μg/g in HEALS. Relative telomere length ranged from 0.22 to 1.78 (median = 0.62) among the 167 participants from HEALS (data not shown). Arsenic concentrations in drinking water among HEALS participants ranged from 0.1 to 864.0 μg/L (median = 50.0 μg/L), that majority of which are far higher than the Maximum Contaminant Levels (MCLs) water established by the U.S. Environmental Protection Agency (10 μg/L, EPA 2001). We do not have water arsenic data for the BEST participants but all BEST samples involved in this study were recuited from the same arears as HEALS.

In analyses of log-transformed creatinine-adjusted urinary total arsenic and expression of telomere-related genes, we identified 9 expression probes (corresponding to 8 genes) with $q < 0.05$, and four of these surpassed the Bonferroni significance threshold ($p < 0.00035$, Table 2, Figure S1–9). This group of significant genes included TERF2 (Figure S1, shelterin pathway), WRN (Figure S2, helicase), DKC1 (Figure S3, regulation of telomerase) and OBFC1 (Figure S4&5, telomere length locus from GWAS). Most of the results above were consistent with the effects of arsenic observed in liver cell lines. Though the samples in the GEO database were quite limited (3 controls Vs. 3 treated with arsenic for each gene), TERF2, RAD51AP1 and MRE11A showed significant associations (p=0.05) and were in the same direction as our results (Supplementary Table S3). The direction of association was

also consistent for WRN and MYC, but these associations were not statistically significant. DKC1 showed an association in the opposite direction with our data, while TERT was not significantly associated with arsenic. In addition, three out of the 143 probes showed associations with BMI after Bonferroni correction (threshold for p = 0.05/143=0.00035). Compared with the underweight group (BMI < 18.5), the participants who were overweight/obese (BMI ≥ 25.0) had down-regulated expression for ERCC1 (β=−0.04, p = 0.00005) and PEILI2 (β=−0.06, p = 0.0002) in contrasting to increased expression of MYC (β=0.08, p = 0.00006).

Supplementary Table S1 lists the results for all 143 probes tested for association with urinary arsenic, including the proportion of samples with a detection p <0.05, an indicator of our ability to detect the presence of a transcript in the RNA samples. Based on these P-values, our platform was unable to detect expression of TERT. Neither probe of TERT, ILMN_2373119 (long isoform) and ILMN_1796005 (short isoform) were detected with p<0.05 in any sample. In addition, arsenic exposure was not associated with expression of ERCC1 or OGG1 in contrast to previous studies (Mo et al., 2009a; Mo et al., 2006). In analyses of the association between telomere-gene expression and SNP-based arsenic methylation capacity, there were no associations passing the FDR correction (q > 0.05) (Supplementary Table S1).

In analyses of non-transformed urinary arsenic and expression of telomere-related genes (Supplementary Table S2), an additional gene (TERF2IP, sherlterin pathway) was found to be statistically significant at P< 0.00035 in addition to TERF2, DKC1, and OBFC1.

Because the effect of arsenic exposure can be modified by arsenic metabolism efficiency, we assessed the interaction between the total urinary arsenic concentrations and SNP-based metabolism efficiency in relation to gene expression. Only one out of the 143 probes was significant at the Bonferroni threshold (p = 0.00035) or FDR correction (q < 0.05): WRN (ILMN_1679881, p for interaction = 0.00008). Associations between urinary arsenic and WRN expression stratified by arsenic methylation capacity (0,1,2,3,4 risk alleles) are shown in Figure 1. Interaction p values for all 143 probes are in supplementary Table S2.

We tested the association between telomere length and arsenic exposure in the 167 samples from HEALS. Telomere length was positively associated with high arsenic exposure (β = 0.12, SE = 0.05, p = 0.02) compared to low arsenic exposure (Table 3, Figure 2). Although not statistically significant in our sample, telomere length was inversely associated with age, as expected. In addition, gender, BMI, smoking and metabolic efficiency did not show association with telomere length (p = 0.85 for rs9527, p= 0.40 for rs11191527 and p = 0.47 for cumulative risk alleles of both SNPs).

4. Discussion

In this population-based study, we assessed the associaton of arsenic exposure with mononuclear cell expression of telomere-related genes and telomere length measured in whole blood. We observed associations between urinary arsenic and expression of several genes with known roles in telomere biology, including TERF2, WRN, DKC1, TERF2IP and

OBFC1. We also observed an interaction between urinary arsenic and arsenic metabolism efficiency in relation to expression of WRN. In addition, we observed that very high arsenic exposure was associated with longer telomeres as measured in whole blood, consistent with a prior research (Li et al., 2012).

In a recent study of arsenic exposure and telomere-related gene expression, Li et al examined associations between urinary arsenic and expression of 62 genes (120 probes) in whole blood among 90 arsenic exposed women in Northern Argentina (Li et al., 2012). Very little evidence of association was observed for the genes analyzed, but Li et al did observe suggestive evidence of a positive association between urinary arsenic and TERT expression, consistent with a prior study of arsenic exposure and TERT expression in whole blood (Mo et al., 2009b).

In this work, we did not detect TERT expression in mononuclear cell RNA, but we do provide evidence that arsenic affects the expression of several telomere-related genes. Urinary arsenic was inversely associated with expression of genes that code for components of the shelterin complex (i.e., TERF2 and TERF2IP) and positively associated with genes involved in repair of telomeric DNA (i.e., RAD51AP1 and MRE11A) and a helicase gene (i.e., WRN). Interestingly, down-regulation of shelterin genes has been suggested to be a cause of telomere damage and dysfunction due to lack of telomere protection (Augereau et al., 2011). Telomere deprotection, specifically, down-regulation of TERF2, may also contribute to activation of “alternative telomere lengthening” (ALT) (Kamranvar et al., 2013), a recombination-based lengthening mechanism that does not involve telomerase and is believed to occur in differentiated cells (Neumann et al., 2013). Urinary arsenic was also positively asssociated with expession of MRE11A, a gene involved in ALT as well as homologous recombination (Escoffier et al., 2005) and WRN, which codes for a helicase protein that is involved in replication of telomereic DNA (including ALT) (Bhattacharyya et al., 2010) and prevention of telomere dysfunction (Crabbe et al., 2004; Eller et al., 2006; Mendez-Bermudez et al., 2012). Expression of WRN was modified significantly by the efficiency of arsenic metablism, suggesting an interaction between arsenic and metabolism efficiency. We provide a summary of potential mechanisms by which arsenic exposure could lengthen telomeres in Figure 3.

The relationship between arsenic exposure and telomere length has been assessed in both exprimental and observational studies. In studies of cell lines (Zhang et al., 2003), animal models (Du et al., 2005), and human cells (Ferrario et al., 2009) high concentrations of As (>1 μM) have been observed to shorten telomeres and reduce cell survival. However, at lower levels (<1 μM), arsenic increases telomerase activity and maintains telomeres, promoting proliferation. A recent population-based study of 202 Nothern Argentina women (Li et al., 2012) showed a positive association between urinary arsenic and telomere length, consistent with our result. While this finding may seem conterintuitive, a recent study of telomere length in yeast suggests that exposure to environmental stressors can shorten or lengthen telomeres, with the direction of the effect being stressor-dependent (Romano et al., 2013). Thus, the direction of the effect of exposures on telomere length may be dependent on both the exposure type and the level of exposure. Thus, arsenic’s carcinogenic mode(s) of action could involve extending telomeres in pre-malignant cells to increase lifespan. Results

from prior studies suggest that arsenic exposure results in longer telomeres due to up-regulation of TERT (Li et al., 2012; Mo et al., 2009b). Unfortunately, expression of neither TERT isoform (ILMN_2373119 or ILMN_1796005) was detected in our study. However, expression of OGG1 (ILMN_1706652 and ILMN_2352609) and ERCC1 (ILMN_1652369, ILMN_1797172 and ILMN_2377496) were detected, and these genes have been reported to be correlated with expression of TERT (Mo et al., 2009b). Consistent with Mo et al (Mo et al., 2009a), OGG1 expression was correlated strongly with all ERCC1 probes ($p < 0.0001$). However, our data showed no association between urinary arsenic and any probes of OGG1 and ERCC1, findings inconsistent with the prior studies (Mo et al., 2009a; Mo et al., 2006).

Discrepancies between our gene expression study and the prior studies by Li et al's (Li et al., 2012) and Mo et al's (Mo et al., 2009a; Mo et al., 2009b; Mo et al., 2006), could be due to a number of factors. First, RNA was extracted from mononuclear cells in our study, but from whole blood in the prior studies. Second, gene expression was assessed using Illumina's HT12 array in the Li H et al's study and in our study, while quantitative real-time qPCR was used by Mo et al. While studies have shown that micro-array-based and qPCR-based expression measures typically show very high correlation, qPCR may have higher sensitivity for detection of low-abundance transcripts. Third, these studies were conducted in different populations, with different levels of arsenic exposure and other environmental and lifestyle factors.

Our gene expression study included 11 genes from regions identified in recent GWA studies of telomere length (Codd et al., 2010; Codd et al., 2013; Gu et al., 2011; Levy et al., 2010; Mangino et al., 2012; Mangino et al., 2009; Prescott et al., 2011). Among these genes, urinary arsenic was inversely associated with OBFC1 and CXCR4 expression and positively associated with PELI2 expression. OBFC1 is a subunit of an alpha accessory factor that stimulates the activity of DNA polymerase-alpha-primase, the enzyme that initiates DNA replication (Casteel et al., 2009). It also appears to function in a telomere-associated complex (Miyake et al., 2009). Although we have little knowledge regarding the roles of the other two genes in the regulation of telomere length, current studies have linked abnormally expressed CXCR4 with some cancers (Sun et al., 2010) and PELI2 with the inflammatory and immune response.

This population-based study of arsenic and telomere-related gene expression is one of the the most comprehensive analysis of telomere-related genes to date, and has a larger sample size than any prior study on this topic. We are the first to examine associations between arsenic exposure and expression of genes at loci kown to harbor genetic variants that influence telomere length. In this analysis, we applied a conservative Bonferroni correction as well as an FDR correction to account for multiple testing. For our analysis of arsenic and telomere length, we selected individuals at the extremes of the exposure distribution to enhance power to detect and assocition. However, our study also has several limitations. The data on expression and telomere length were obtained from two different cohorts, thus, we were unable to examine the association between telomere length and gene expression. Our analysis of gene expression is based on the assumption that the effects of arsenic on expression of telomere-related genes can be detected in peripheral mononuclear cells. While much of the telomere maintenance relevant to mononuclear cells is believed to occur in

hematopoietic stem cells and precursor cells, it is possible that arsenic may impact telomeres and telomere maintenance in differentiated cells (Hodes et al., 2002). Furthermore, we are unable to assess expression for transcripts expressed at levels too low to be detected using the Illumina array (e.g. TERT). In our analyses of arsenic metabolism efficiency and gene expression, we did not have data on urinary arsenic metabolites, but we used metabolism-related variants in the AS3MT region (Pierce et al., 2012) as a proxy for metabolism. Similarly, we lacked data on urinary arsenic metabolites for the majority of the participants with telomere length data, and no association was observed between telomere length and the two *AS3MT* SNPs.

While our results need confirmation based on large independent studies, these findings, taken together, provide suggestive evidence that arsenic's carcinogenic mode of action may involve alteration of the telomere maintenance and/or telomere damage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Ahsan H, et al. Arsenic metabolism, genetic susceptibility, and risk of premalignant skin lesions in Bangladesh. Cancer Epidemiol Biomarkers Prev. 2007; 16:1270–1278. [PubMed: 17548696]

Ahsan H, et al. Health Effects of Arsenic Longitudinal Study (HEALS): description of a multidisciplinary epidemiologic investigation. J Expo Sci Environ Epidemiol. 2006; 16:191–205. [PubMed: 16160703]

Argos M, et al. Arsenic exposure from drinking water, and all-cause and chronic-disease mortalities in Bangladesh (HEALS): a prospective cohort study. Lancet. 2010; 376:252–258. [PubMed: 20646756]

Argos M, et al. Baseline comorbidities in a skin cancer prevention trial in Bangladesh. Eur J Clin Invest. 2013; 43:579–588. [PubMed: 23590571]

Augereau A, et al. Telomeric damage in early stage of chronic lymphocytic leukemia correlates with shelterin dysregulation. Blood. 2011; 118:1316–1322. [PubMed: 21355086]

Barbosa-Morais NL, et al. A re-annotation pipeline for Illumina BeadArrays: improving the interpretation of gene expression data. Nucleic Acids Res. 2010; 38:e17. [PubMed: 19923232]

Basu A, et al. Genetic toxicology of a paradoxical human carcinogen, arsenic: a review. Mutat Res. 2001; 488:171–194. [PubMed: 11344043]

Bhattacharjee P, et al. Systems biology approaches to evaluate arsenic toxicity and carcinogenicity: An overview. Int J Hyg Environ Health. 2013

Bhattacharyya S, et al. Unwinding protein complexes in ALTernative telomere maintenance. J Cell Biochem. 2010; 109:7–15. [PubMed: 19911388]

Butler RN, et al. Biomarkers of aging: from primitive organisms to humans. J Gerontol A Biol Sci Med Sci. 2004; 59:B560–B567. [PubMed: 15215265]

Casteel DE, et al. A DNA polymerase-{alpha}{middle dot}primase cofactor with homology to replication protein A-32 regulates DNA replication in mammalian cells. J Biol Chem. 2009; 284:5807–5818. [PubMed: 19119139]

Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res. 2002; 30:e47. [PubMed: 12000852]

Cawthon RM, et al. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet. 2003; 361:393–395. [PubMed: 12573379]

Celik I, et al. Arsenic in drinking water and lung cancer: a systematic review. Environ Res. 2008; 108:48–55. [PubMed: 18511031]

Chatterjee D, et al. Arsenic exposure through drinking water leads to senescence and alteration of telomere length in humans: A case-control study in West Bengal, India. Mol Carcinog. 2014

Chen CJ, et al. Cancer potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking water. Br J Cancer. 1992; 66:888–892. [PubMed: 1419632]

Codd V, et al. Common variants near TERC are associated with mean telomere length. Nat Genet. 2010; 42:197–199. [PubMed: 20139977]

Codd V, et al. Identification of seven loci affecting mean telomere length and their association with disease. Nat Genet. 2013; 45:422–427. 427e1–427e2. [PubMed: 23535734]

Crabbe L, et al. Defective telomere lagging strand synthesis in cells lacking WRN helicase activity. Science. 2004; 306:1951–1953. [PubMed: 15591207]

Du CW, et al. Latent membrane protein-1 of Epstein - Barr virus increases sensitivity to arsenic trioxide-induced apoptosis in nasopharyngeal carcinoma cell. Exp Oncol. 2005; 27:267–272. [PubMed: 16404345]

Eller MS, et al. A role for WRN in telomere-based DNA damage responses. Proc Natl Acad Sci U S A. 2006; 103:15073–15078. [PubMed: 17015833]

Escoffier E, et al. A balanced transcription between telomerase and the telomeric DNA-binding proteins TRF1, TRF2 and Pot1 in resting, activated, HTLV-1-transformed and Tax-expressing human T lymphocytes. Retrovirology. 2005; 2:77. [PubMed: 16354306]

Ferrario D, et al. Arsenic induces telomerase expression and maintains telomere length in human cord blood cells. Toxicology. 2009; 260:132–141. [PubMed: 19464579]

Finkel T, et al. The common biology of cancer and ageing. Nature. 2007; 448:767–774. [PubMed: 17700693]

Fitzpatrick AL, et al. Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. Am J Epidemiol. 2007; 165:14–21. [PubMed: 17043079]

Gu J, et al. A genome-wide association study identifies a locus on chromosome 14q21 as a predictor of leukocyte telomere length and as a marker of susceptibility for bladder cancer. Cancer Prev Res (Phila). 2011; 4:514–521. [PubMed: 21460395]

Han J, et al. A prospective study of telomere length and the risk of skin cancer. J Invest Dermatol. 2009; 129:415–421. [PubMed: 18668136]

Hodes RJ, et al. Telomeres in T and B cells. Nat Rev Immunol. 2002; 2:699–706. [PubMed: 12209138]

Hughes MF. Arsenic toxicity and potential mechanisms of action. Toxicol Lett. 2002; 133:1–16. [PubMed: 12076506]

Hughes MF, et al. Arsenic exposure and toxicology: a historical perspective. Toxicol Sci. 2011; 123:305–332. [PubMed: 21750349]

Kamranvar SA, et al. Telomere dysfunction and activation of alternative lengthening of telomeres in B-lymphocytes infected by Epstein-Barr virus. Oncogene. 2013; 32:5522–5530. [PubMed: 23708666]

Kawata K, et al. Classification of heavy-metal toxicity by human DNA microarray analysis. Environ Sci Technol. 2007; 41:3769–3774. [PubMed: 17547211]

Kitchin KT. Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicol Appl Pharmacol. 2001; 172:249–261. [PubMed: 11312654]

Levy D, et al. Genome-wide association identifies OBFC1 as a locus involved in human leukocyte telomere biology. Proc Natl Acad Sci U S A. 2010; 107:9293–9298. [PubMed: 20421499]

Li H, et al. Arsenic exposure through drinking water is associated with longer telomeres in peripheral blood. Chem Res Toxicol. 2012; 25:2333–2339. [PubMed: 22917110]

Liu J, Waalkes MP. Liver is a target of arsenic carcinogenesis. Toxicol Sci. 2008; 105:24–32. [PubMed: 18566022]

Liu L, et al. Oxidative stress contributes to arsenic-induced telomere attrition, chromosome instability, and apoptosis. J Biol Chem. 2003; 278:31998–32004. [PubMed: 12767976]

Lu J, Liu Y. Deletion of Ogg1 DNA glycosylase results in telomere base damage and length alteration in yeast. EMBO J. 2010; 29:398–409. [PubMed: 19942858]

Mangino M, et al. Genome-wide meta-analysis points to CTC1 and ZNF676 as genes regulating telomere homeostasis in humans. Hum Mol Genet. 2012; 21:5385–5394. [PubMed: 23001564]

Mangino M, et al. A genome-wide association study identifies a novel locus on chromosome 18q12.2 influencing white cell telomere length. J Med Genet. 2009; 46:451–454. [PubMed: 19359265]

McEachern MJ, et al. Telomeres and their control. Annu Rev Genet. 2000; 34:331–358. [PubMed: 11092831]

Mendez-Bermudez A, et al. The roles of WRN and BLM RecQ helicases in the Alternative Lengthening of Telomeres. Nucleic Acids Res. 2012; 40:10809–10820. [PubMed: 22989712]

Mink PJ, et al. Low-level arsenic exposure in drinking water and bladder cancer: a review and meta-analysis. Regul Toxicol Pharmacol. 2008; 52:299–310. [PubMed: 18783726]

Mirabello L, et al. Worldwide genetic structure in 37 genes important in telomere biology. Heredity (Edinb). 2012; 108:124–133. [PubMed: 21731055]

Mirabello L, et al. The association of telomere length and genetic variation in telomere biology genes. Hum Mutat. 2010; 31:1050–1058. [PubMed: 20597107]

Miyake Y, et al. RPA-like mammalian Ctc1-Stn1-Ten1 complex binds to single-stranded DNA and protects telomeres independently of the Pot1 pathway. Mol Cell. 2009; 36:193–206. [PubMed: 19854130]

Mo J, et al. Elevated ERCC1 gene expression in blood cells associated with exposure to arsenic from drinking water in Inner Mongolia. Anticancer Res. 2009a; 29:3253–3259. [PubMed: 19661343]

Mo J, et al. Elevated human telomerase reverse transcriptase gene expression in blood cells associated with chronic arsenic exposure in Inner Mongolia, China. Environ Health Perspect. 2009b; 117:354–360. [PubMed: 19337508]

Mo J, et al. Chronic arsenic exposure and oxidative stress: OGG1 expression and arsenic exposure, nail selenium, and skin hyperkeratosis in Inner Mongolia. Environ Health Perspect. 2006; 114:835–841. [PubMed: 16759981]

Nan H, et al. Shorter telomeres associate with a reduced risk of melanoma development. Cancer Res. 2011a; 71:6758–6763. [PubMed: 22028319]

Nan H, et al. Genetic variants in telomere-maintaining genes and skin cancer risk. Hum Genet. 2011b; 129:247–253. [PubMed: 21116649]

Nermell B, et al. Urinary arsenic concentration adjustment factors and malnutrition. Environ Res. 2008; 106:212–218. [PubMed: 17900556]

Neumann AA, et al. Alternative lengthening of telomeres in normal mammalian somatic cells. Genes Dev. 2013; 27:18–23. [PubMed: 23307865]

Nixon DE, et al. Total arsenic in urine: palladium-persulfate vs nickel as a matrix modifier for graphite furnace atomic absorption spectrophotometry. Clin Chem. 1991; 37:1575–1579. [PubMed: 1893592]

Olovnikov AM. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. J Theor Biol. 1973; 41:181–190. [PubMed: 4754905]

Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001; 29:e45. [PubMed: 11328886]

Pierce BL, et al. Genome-wide association study identifies chromosome 10q24.32 variants associated with arsenic metabolism and toxicity phenotypes in Bangladesh. PLoS Genet. 2012; 8:e1002522. [PubMed: 22383894]

Prescott J, et al. Genome-wide association study of relative telomere length. PLoS One. 2011; 6:e19635. [PubMed: 21573004]

Ravenscroft, P., et al. Arsenic Pollution: A Global Synthesis. Wiley-Blackwell; 2009.

Romano GH, et al. Environmental stresses disrupt telomere length homeostasis. PLoS Genet. 2013; 9:e1003721. [PubMed: 24039592]

Sanders JL, et al. Leukocyte telomere length is associated with noninvasively measured age-related disease: The Cardiovascular Health Study. J Gerontol A Biol Sci Med Sci. 2012; 67:409–416. [PubMed: 21934123]

Schoen A, et al. Arsenic toxicity at low doses: epidemiological and mode of action considerations. Toxicol Appl Pharmacol. 2004; 198:253–267. [PubMed: 15276404]

Shen M, et al. A prospective study of telomere length measured by monochrome multiplex quantitative PCR and risk of lung cancer. Lung Cancer. 2011; 73:133–137. [PubMed: 21507503]

Stewart SA, Weinberg RA. Telomerase and human tumorigenesis. Semin Cancer Biol. 2000; 10:399–406. [PubMed: 11170862]

Storey JD. The positive false discovery rate: a Bayesian interpretation and the q-value. Annals of Statistics. 2003; 31:2013–2035.

Sun X, et al. CXCL12 / CXCR4 / CXCR7 chemokine axis and cancer progression. Cancer Metastasis Rev. 2010; 29:709–722. [PubMed: 20839032]

van Geen A, et al. Spatial variability of arsenic in 6000 tube wells in a 25 km(2) area of Bangladesh. Water Resources Research. 2003; 39

Vannier JB, et al. ERCC1/XPF protects short telomeres from homologous recombination in Arabidopsis thaliana. PLoS Genet. 2009; 5:e1000380. [PubMed: 19214203]

Yu HS, et al. Arsenic carcinogenesis in the skin. J Biomed Sci. 2006; 13:657–666. [PubMed: 16807664]

Yuan Y, et al. Kidney cancer mortality: fifty-year latency patterns related to arsenic exposure. Epidemiology. 2010; 21:103–108. [PubMed: 20010213]

Zhang TC, et al. Effects of arsenic on telomerase and telomeres in relation to cell proliferation and apoptosis in human keratinocytes and leukemia cells in vitro. Carcinogenesis. 2003; 24:1811–1817. [PubMed: 12919960]

Zimmermann S, Martens UM. Telomeres, senescence, and hematopoietic stem cells. Cell Tissue Res. 2008; 331:79–90. [PubMed: 17960423]

Highlights

- Urinary arsenic was associated with expression of multiple telomere-related genes.
- High arsenic exposure was associated with longer telomeres.
- Arsenic's association with WRN expression was modified by arsenic metabolism status.
- Arsenic's carcinogenic mechanism may involve telomere dysfunction.

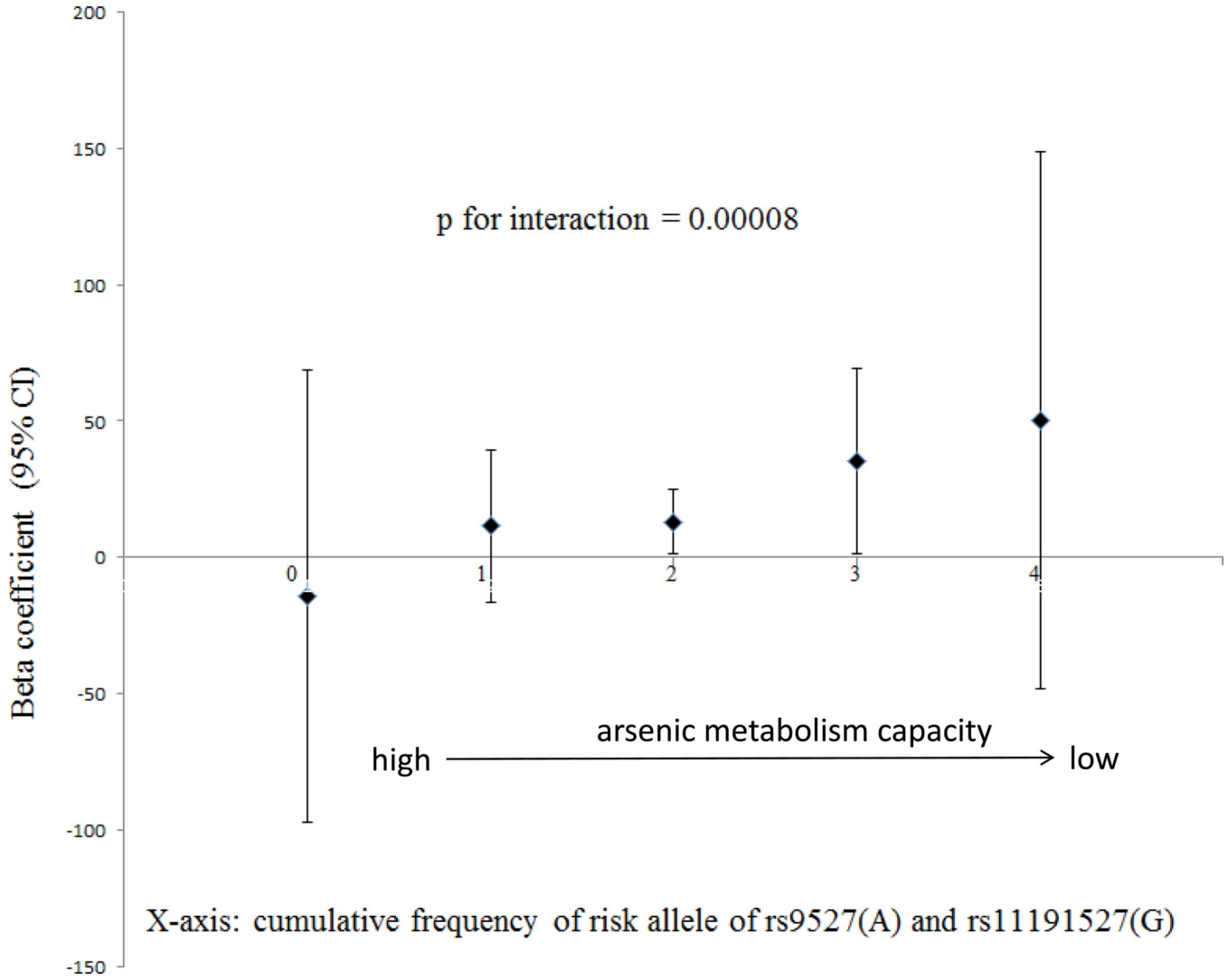

Fig. 1. Associations between urinary arsenic and expression of WRN (ILMN_1679881), stratified by arsenic metabolism capacity

The p for interaction between urinary arsenic and arsenic metabolism efficiency in relation to expression of WRN (ILMN_1679881) was obtained from a linear regression model, adjusting for sex, age, experimental batch, smoking status and BMI. Diamond shapes and error bars denote beta coefficients and 95% confidence intervals (95% CI) derived from linear regression model stratified by arsenic metabolism efficiency. Arsenic metabolism efficiency was represented by the cumulative frequency (0,1,2,3,4) of risk alleles of rs9527(A) and rs11191527(G). Arsenic metabolism capacity ranges from from high (0) to low (4).

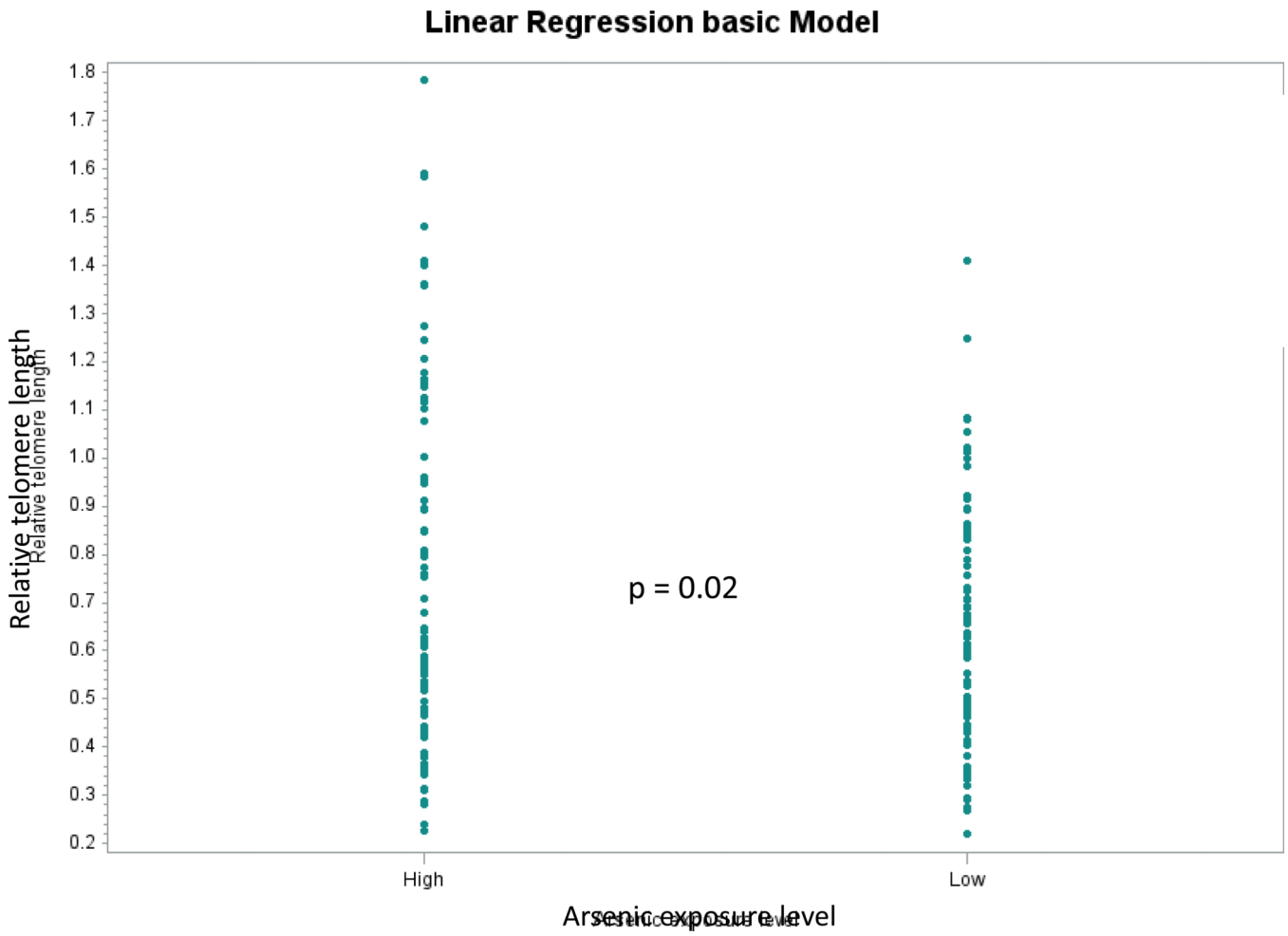

Fig. 2. Relationship between arsenic exposure status and telomere length

P-value was obtained from a linear regression model adjusted for age, sex, smoking, and BMI.

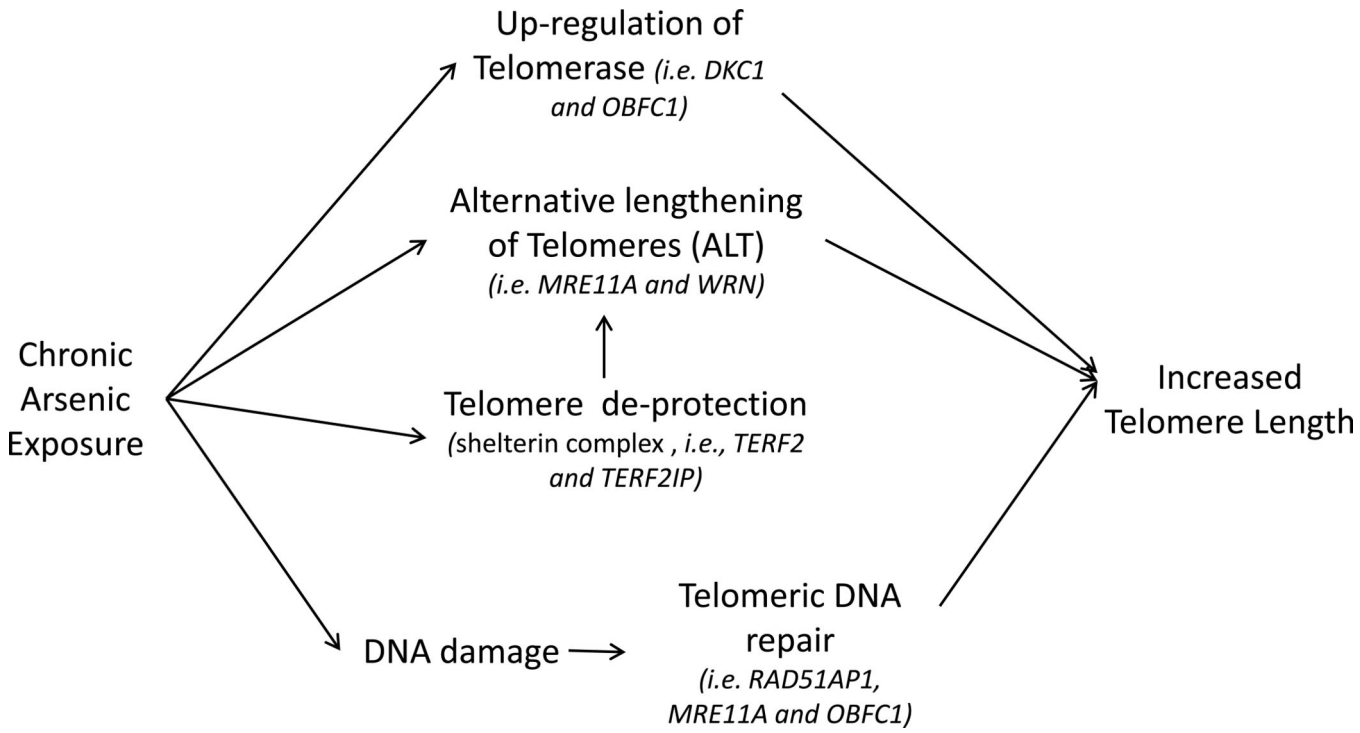

Fig. 3. A summary of potential mechanisms by which arsenic exposure could lengthen telomeres

Examples of genes showing association with arsenic exposure are provided.

Table 1

Characteristics of individuals participating in BEST (gene expression study) and HEALS (telomere length study) [a]

	Expression study (BEST Cohort)	Telomere length study (HEALS Cohort)
N	1,799	167
Gender (%)		
Men	991 (55.1)	63 (37.7)
Women	808 (44.9)	104 (62.3)
Age	43.5 ± 10.6	35.5 ± 9.7
Smoking status		
Never	1095 (60.9)	116 (69.5)
Former	186 (10.3)	13 (7.8)
current	518 (28.8)	38 (22.8)
BMI		
Underweight (<18.5)	685 (38.1)	72 (43.1)
Normal (18.5–24.9)	951 (52.9)	83 (49.7)
Overweight/obese (≥25.0)	163 (9.1)	12 (7.2)
Urinary arsenic (μg/g)	336.7 ± 437.7	Low[b]: 61.9 ± 23.7 High[b]: 856.0 ± 913.1
Water arsenic (μg/L)	N/A	Low[b]: 3.6 ± 3.4 High[b]: 288.0 ± 108.6

[a] Categorical variables were shown in counts and percentages; continuous variables in means±standard deviation (SD).

[b] Participants were selected from the extremes of the arsenic exposure. High exposure was defined as arsenic concentration >145 μg/L in water and >339 μg/g in urine (measured at baseline). Low exposure was defined as <12 μg/L in water and <107 μg/g in urine.

Table 2

Association between urinary arsenic concentrations (log-transformed) and expression of telomere-related genes

probeID	gene	chr	Beta[a]	SE	p[b]	Q[b]
ILMN_1768488	TERF2	chr16	−0.02	0.005	7.1×10^{-6}	0.0007
ILMN_1679881	WRN	chr8	0.02	0.005	2.1×10^{-5}	0.001
ILMN_1671257	DKC1	chrX	−0.02	0.005	0.0002	0.008
ILMN_1906158	OBFC1[c]	chr10	−0.01	0.004	0.0003	0.008
ILMN_1670353	RAD51AP1	chr12	0.02	0.005	0.0005	0.01
ILMN_1760802	MRE11A	chr11	0.02	0.005	0.0008	0.01
ILMN_1789186	OBFC1[c]	chr10	−0.02	0.005	0.001	0.02
ILMN_2110908	MYC	chr8	−0.04	0.01	0.002	0.02
ILMN_1680618	MYC	chr8	−0.03	0.01	0.004	0.04

[a] Beta coefficients and standard Errors (SE) were obtained from linear regression models adjusted for age, sex, experimental batch, smoking, and BMI.

[b] P: uncorrected p-value (threshold for Bonferroni Correction: p = 0.00035); Q: q-value based on FDR value (threshold for FDR: q = 0.05)

[c] reported genes from recent GWA studies of telomere length

Table 3

Association of urinary arsenic concentrations and participant characteristics with telomere length[a]

	N	Beta	SE	p-value
Arsenic exposure				
low	83	referent		
high	84	0.12	0.05	0.02
Sex				
female	104	referent		
male	63	0.04	0.08	0.63
BMI				
Underweight (<18.5)	72	referent		
Normal (18.5–24.9)	83	0.03	0.05	0.63
Overweight/obese (≥25.0)	12	−0.04	0.11	0.69
Age				
18 ~ 30	55	referent		
~ 40	68	0.008	0.06	0.89
~ 50	34	−0.03	0.08	0.70
~ 62	10	−0.11	0.11	0.33
Smoking status				
Never	116	referent		
Ever	51	−0.06	0.08	0.47

[a]Beta coefficients and standard errors were obtained from linear regression models adjusting for age, sex, smoking, and BMI.