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Arsenic Exposure through Drinking Water Is Associated with Longer Telomeres in Peripheral Blood

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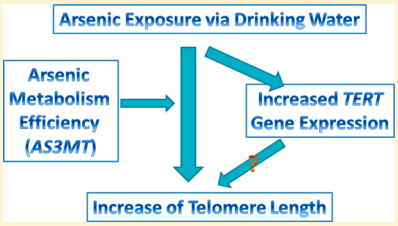
ABSTRACT: Inorganic arsenic is a strong carcinogen, possibly by interaction with the telomere length. The aim of the study was to evaluate how chronic arsenic exposure from drinking water as well as the arsenic metabolism efficiency affect the individual telomere length and the expression of telomere-related genes. Two hundred two women with a wide range in exposure to arsenic via drinking water (3.5−200 μg/L) were recruited. Concentrations of arsenic metabolites in urine [inorganic arsenic (iAs), methylarsonic acid (MMA), and dimethylarsinic acid (DMA)] were measured. The relative telomere length in blood was measured by quantitative real-time polymerase chain reaction. Genotyping (N = 172) for eight SNPs in *AS3MT* and gene expression of telomere-related genes (in blood; N = 90) were performed. Urinary arsenic (sum of metabolites) was positively associated with telomere length ($\beta = 0.65 \times 10^{-4}$, 95% CI = 0.031×10^{-4}−1.3×10^{-4}, adjusted for age and BMI). Individuals with above median fractions of iAs and MMA showed significantly longer telomeres by increasing urinary arsenic ($\beta = 1.0 \times 10^{-4}$, 95% CI = 0.21×10^{-4}−1.8×10^{-4} at high % iAs; $\beta = 0.88 \times 10^{-4}$ 95% CI = 0.12×10^{-4}−1.6×10^{-4} at high % MMA) than those below the median (p = 0.80 and 0.44, respectively). Similarly, carriers of the slow and more toxic metabolizing *AS3MT* haplotype showed stronger positive associations between arsenic exposure and telomere length, as compared to noncarriers (interaction urinary arsenic and haplotype p = 0.025). Urinary arsenic was positively correlated with the expression of telomerase reverse transcriptase (*TERT*, Spearman r = 0.22, p = 0.037), but no association was found between *TERT* expression and telomere length. Arsenic in drinking water influences the telomere length, and this may be a mechanism for its carcinogenicity. A faster and less toxic arsenic metabolism diminishes arsenic-related telomere elongation.



INTRODUCTION

Telomeres are formed by tandem repeats (TTAGGG) located at the end of each eukaryotic chromosome. They are shortened for each cell division due to "the end replication problem".[1] Telomeres are responsible for regulation of cellular life span. At a certain length, the telomeres signal the cell to stop dividing, since too short telomeres may cause genomic instability that accelerates the accumulation of genetic changes responsible for tumorigenesis.[2−5] Short telomeres in peripheral blood have repeatedly been reported as a risk marker for several cancer types.[6−9] Telomere maintenance is the primary mechanism by which cancer cells overcome mortality and extend their life span,[2,4] which is mainly sustained by the activation of the telomere-elongating protein telomerase reverse transcriptase (encoded by the gene *TERT*).

Environmental carcinogens may influence the telomere length,[10,11] but there are few human studies on arsenic exposure. Arsenic in drinking water is a major public health problem in several parts of the world, in particular Bangladesh, India, China, Argentina, and the United States.[12] Arsenic is a potent carcinogen, and chronic exposure increases the risk of cancer in the skin, urinary bladder, lung, and possibly in the kidney, liver, and the prostate. However, arsenic is not a strong direct mutagen, and several other modes of action for arsenic carcinogenesis have been suggested.[13−16] There is some support that arsenic alters the telomeres: arsenic increased telomere attrition, chromosomal rearrangements, and apoptotic cell death in mouse embryos with short telomeres.[17] On the other hand, in vitro studies showed that arsenic increased the activity of *TERT*.[18−20] Also, in people exposed to arsenic via drinking water (1−1000 μg/L) in Inner Mongolia, *TERT* expression was positively associated with both arsenic concentrations in water and in nails and the severity of hyperkeratosis, a common arsenic-related skin lesion.[19] These apparently contrasting effects of arsenic on telomere length may be related to the arsenic dose. In human cord blood cells, sub-nanomolar arsenite was found to increase *TERT* gene and protein expression in vitro, resulting in maintained telomere length, while at 1 μM concentrations, the *TERT* expression and telomere length decreased.[18]

Also, the form of arsenic and the metabolism in the body may be influential. Inorganic arsenic (iAs) is metabolized in humans by a series of reduction and methylation reactions that produce methylarsonic acid (MMA) and dimethylarsinic acid (DMA). The addition of two methyl groups to form DMA from the highly reactive arsenic form arsenite (AsIII), via the even more reactive MMAIII, results in the formation of a much less reactive compound, which is rapidly excreted in urine.[21] Thus, a higher fraction of MMA and a lower fraction of DMA

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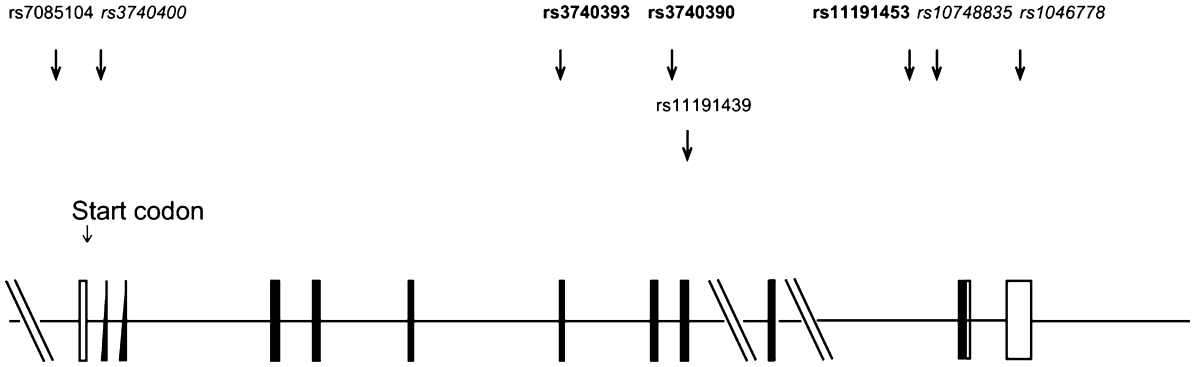

Figure 1. *AS3MT* gene and polymorphisms under study. The black line shows the 5′- and 3′-flanking regions and the intronic regions. Translated exons are illustrated as black boxes, while untranslated regions are illustrated as white boxes. Polymorphisms are illustrated by arrows pointing at their positions in the gene. Inclined lines are shown when parts of the intronic or 5′-flanking regions are shortened for the sake of simplicity.

in urine are associated with a lower rate of arsenic excretion in urine[21,22] and more toxic effects.[23,24] The main methyltransferase in arsenic metabolism is arsenic (+3 oxidation state) methyltransferase (AS3MT),[25] which can methylate both iAs and MMA. We have previously shown that the *AS3MT* genotype is the main determinant of the efficiency of the metabolism of arsenic.[26]

The aim of present study was to clarify how human exposure to arsenic in drinking water affects the telomere length and expression of telomere-related genes and whether the effect is modified by the arsenic metabolism efficiency. Our main hypothesis was that arsenic influences the telomere length through action on expression of *TERT*. However, arsenic may also act through other mechanisms; one plausible is that arsenic acts on expression of telomere binding proteins that stabilize the telomere structure.

MATERIALS AND METHODS

Study Site and Population. We studied 202 women living on the Andean plateau (3800 m above sea level) in Northern Argentina, an area that has minimal industrial and vehicle-derived environmental pollution. However, some villages have elevated concentrations of arsenic in drinking water.[27] The recruitment of the study participants and their characteristics have been described previously in detail.[26,28] In short, the study participants were recruited with the help of the local health clinics. Most of the study participants (N = 161) were from the village San Antonio de los Cobres with about 5000 inhabitants and 200 μg/L arsenic in the public drinking water. The other participants (N = 41) were from three small surrounding villages with lower concentrations of arsenic in the water (3.5−73 μg/L).

The interviews revealed that almost all women drank public drinking water exclusively and that their diets consisted mainly of corn, beans, chicken, and pork. Four women reported that they smoked cigarettes, one reported drinking alcohol, and almost half of the women (46.5%) reported that they often chewed coca leaves. Only three women reported taking any medication at the time of the study; one was being treated for gastritis, and two were being treated for hypertension. The women were asked if they had had any diseases, and their hands were inspected for arsenic-related skin lesions.

Biological samples were collected during the daytime as nonfasting spot samples. Peripheral blood for DNA extraction (N = 202) was collected in K_2EDTA tubes (Vacuette; Greiner, Germany), and blood for RNA extraction (N = 122; the first women recruited to the study) was collected in PAX tubes (Beckton Dickinson, Franklin Lakes, NJ). Spot urine samples were collected and processed as described previously.[29] Blood and urine samples were kept at −20 °C before and after transport (with cooling blocks) to Sweden for analysis. Informed consent, both oral and written, was provided by all of the study participants. The study was approved by the Ministry of Health in Salta, Argentina, and the Regional Ethical Committee of Karolinska Institutet, Sweden.

Analysis of Arsenic in Urine. The concentrations of arsenic metabolites, that is, iAs, MMA, and DMA, were measured using high-performance liquid chromatography coupled with hydride generation and inductively coupled plasma mass spectrometry (Agilent 7500ce; Agilent Technologies, Tokyo, Japan), employing appropriate quality control.[30] The sum of metabolite concentrations (hereafter called urinary arsenic) was used to assess arsenic exposure. The fractions of the different metabolites in urine (percentages of urinary arsenic) were used to assess the efficiency of arsenic metabolism. All measured concentrations of elements in urine were adjusted to the mean specific gravity of urine (1.020 g/mL), determined by a digital refractometer (EUROMEX RD 712 clinical refractometer; EUROMEX, Arnhem, The Netherlands).[17]

Telomere Length Determination and Genotyping. DNA was isolated from whole blood by Qiagen DNA Blood Mini kit (QIAGEN, Heidelberg, Germany). Genotyping for eight *AS3MT* polymorphisms that have been associated with arsenic metabolism[26] (Figure 1 shows the *AS3MT* structure and the positions of the polymorphisms genotyped) was performed using Sequenom (San Diego, CA) technology by Swegenés DNA Facility at Malmö University Hospital, Malmö, Sweden. Haplotypes from the genotypes of these polymorphisms were inferred using PHASE software.[31] The two major *AS3MT* haplotypes in this population were evaluated here: (1) AAGGTTGT (high fraction of MMA and low fraction of DMA, i.e., a slow and more toxic metabolism, haplotype frequency = 26%), and (2) GCCATCAC (low % MMA and high DMA, i.e., a faster and less toxic metabolism, haplotype frequency = 70%), where the order of the polymorphisms in *AS3MT* is (5′-3′): rs7085104, rs3740400, rs3740393, rs3740390, rs11191439, rs11191453, rs10748835, and rs1046778.[26]

For nine individuals, there was not enough DNA for telomere length determination. Relative telomere length quantification was determined by quantitative polymerase chain reaction (PCR) as described in detail,[32] based on the method reported by Cawthon.[33] In short, an aliquot of 6 μL of sample DNA (3 ng/μL) was added to each reaction (end volume, 20 μL). A standard curve, a reference DNA, and a negative control were included in each run. For each standard curve, one calibrator DNA sample was diluted serially by 2-fold per dilution to produce five concentrations of 0.625−10 ng/μL. Each sample, standard curve, reference, and negative was run in triplicate. Master mixes were prepared, containing 0.5U *Taq* Platina (Invitrogen, Carlsbad, CA), 1 × PCR buffer, 0.8 mM dNTPs, 1.75 mM $MgCl_2$, 0.3 mM SybrGreen I (Invitrogen), 1 × Rox (Invitrogen), and either telomere primers (0.45 μM of each primer), or hemoglobin β chain (*HBG*) primers (0.45 μM for each primer). The PCR was performed on a real-time PCR machine (7900HT, Applied Biosystems). The R^2 for each standard curve was >0.99. Standard deviations (for Ct values) were accepted at <0.2. The relative length of the telomeres was obtained through calculating the ratio (T/S) of telomere repeats

product and single copy gene product (*HBG*) for each individual, by the formula $T/S = 2^{-Ct}$, where $Ct = Ct_{telomere} - Ct_{HBG}$. This ratio was then compared with the ratio of the reference DNA. The telomere length ratio is unitless. The reference samples were included in each run and demonstrated a CV of 10%, based on eight runs.

Gene Expression Analysis. RNA was extracted from whole blood with the PAXgene Blood RNA kit (PreAnalytiX) and stored in −80 °C. The RNA concentration and purity were measured by a Nanodrop spectrophotometer (Wilmington, DE), and the RNA integrity (RIN) was evaluated on a Bioanalyzer 2100 (Agilent, CA), showing a good quality of the RNA (RIN > 7.5). For the gene expression analysis, 90 individuals were chosen with a wide range of urinary arsenic (10−1251 μg/L), which were matched for age, weight, and body mass index (BMI). For the whole genome gene expression analysis, DirectHyb HumanHT-12 v4.0 (Illumina, San Diego, CA) was used according to the manufacturer's instructions, and the analysis was performed at SCIBLU core facility at Lund University. Background signals were filtered from the gene expression data by BioArray Software Environment (BASE).[34] Gene expression data for telomere-related genes selected based on literature searches were included in the analyses (120 transcripts from 62 genes). Eighty-five of the 90 individuals had data on telomere length.

Statistical Analysis. Correlations between variables were analyzed by Spearman's ρ correlation (r_s). The linearity of the relationship of arsenic exposure (measured as urinary arsenic) and telomere length was evaluated by visual inspection of a scatter plot (Figure 2), quintiles of urinary arsenic, and analyzing the residuals by P−P plots. Association between telomere length and urinary arsenic was then analyzed by linear regression analysis. The models were adjusted for age and BMI, which were statistically significantly associated with the telomere length in the Spearman's correlation analysis.

To evaluate potential effects of arsenic metabolism on the telomere length, we stratified the associations between arsenic in urine and telomere length for the metabolite fraction (median split). We also evaluated the effect of the major arsenic metabolizing gene *AS3MT* on the association between arsenic and telomere length (first-degree relatives were excluded, total $N = 166$ with telomere length data) by multivariate regression analysis where we included haplotype (as categorical variable), urinary arsenic, and an interaction term between haplotype and urinary arsenic. To obtain an effect estimate in carriers with different numbers of haplotype copies, we performed regression analysis of urinary arsenic and telomere length, stratified by *AS3MT* haplotype. All analyses were adjusted for age and BMI.

Spearman's correlation was adopted to investigate the correlation between urinary arsenic and expression levels of telomere-related genes as well as the association between telomere length and expression levels of telomere-related genes. The p values were adjusted for false discovery rate (FDR) by the approach reported by Benjamini and Hochberg.[35] All statistical analyses were completed using SPSS 18.0 (SPSS Inc., Chicago, IL), except for the FDR adjustments, which were made using R (2.15.1). Statistical significance refers to $P < 0.05$ (two-tailed).

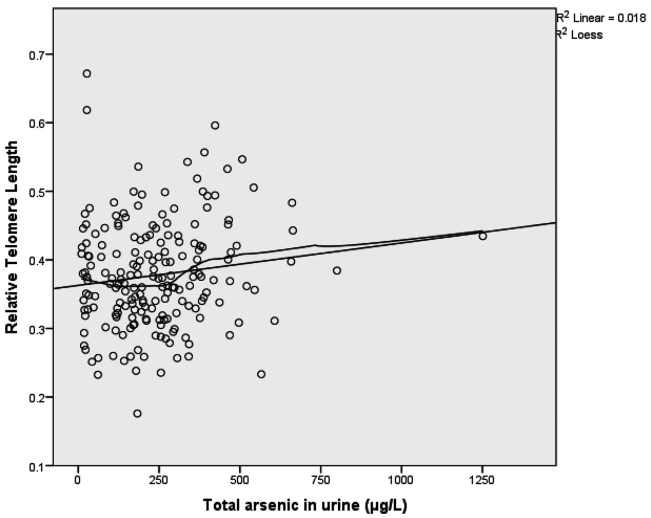

Figure 2. Scatter plot that shows the association between arsenic concentration in urine (μg/L, adjusted to specific gravity 1.020 g/mL) and the telomere length in blood (arbitrary unit). The linear fit line and loess fit line are presented.

RESULTS

The participant characteristics, the concentrations of arsenic metabolites in urine, and relative telomere length are listed in Table 1. None of the women showed any sign of arsenic-related hyperkeratosis on their hands or reported any history of malignancy. There was a wide range in urinary arsenic concentrations (median = 230 μg/L, range = 0.1−1251 μg/L), whereas the relative telomere length varied more than 3-fold (median = 0.37, range = 0.18−0.67). The telomere length was inversely correlated with age and BMI and nonsignificantly positively related with urinary arsenic ($p = 0.21$; Table 1). However, it was positively associated with the fraction of iAs in urine and inversely associated with the fraction of DMA ($p = 0.030$ and 0.013, respectively). BMI increased with increasing age ($r_s = 0.46$, $p < 0.01$). In the subgroup of women analyzed for gene expression in peripheral blood, the correlations between the telomere length and the participant characteristics were similar; however, the correlations with urinary arsenic metabolite fractions were weaker and nonsignificant.

A linear relationship was found between arsenic in urine and telomere length. In the linear regression model, urinary arsenic showed significant positive association with telomere length [$\beta = 0.65 \times 10^{-4}$, 95% confidence interval (CI) 0.031×10^{-4}−1.3

Table 1. Characteristics of the Study Participants, Concentrations of Arsenic in Urine, And Relative Telomere Length in Blood[a]

	$N = 202$[b]			$N = 90$[b]		
variables	median	range	r_s[c]	median	range	r_s[c]
age (years)	34	12−80	−0.34**	32	12−65	−0.28**
BMI (kg/m^2)	24.7	16.4−40	−0.28**	24.0	16.4−35.7	−0.32**
urinary arsenic (μg/L)[d]	230	10.1−1251	0.13	203	10.1−1251	0.14
fraction of iAs (%)	11.6	2.3−34.2	0.16*	13.0	3.3−32.9	0.096
fraction of MMA (%)	7.7	2.4−22.1	0.020	7.7	2.4−18.5	0.021
fraction of DMA (%)	80.2	56.4−95.1	−0.18*	78.8	56.4−93.5	−0.13
relative telomere length	0.37	0.18−0.67		0.38	0.24−0.62	

[a]Also, associations of each variable (Spearman correlation, r_s) with the telomere length are shown. [b]$N = 202$ whole study population, and $N = 90$ subgroup analyzed for expression of telomere-related genes in whole blood. [c]*$p < 0.05$; **$p < 0.01$. [d]Adjusted for the specific gravity of urine (1.020 g/mL).

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$\times 10^{-4}$; Table 2 and Figure 2]. There were two outliers with long telomere length and low arsenic exposure, and when excluding them, the associations between urinary arsenic and telomere length became stronger ($\beta = 0.87 \times 10^{-4}$, 95% CI 0.30×10^{-4}–1.4×10^{-4}); there was also one outlier with high urinary arsenic (urinary arsenic = 1251 μg/L), but the effect estimate was similar when excluding this individual ($\beta = 0.72 \times 10^{-4}$, 95% CI 0.039×10^{-4}–1.4×10^{-4}). These three outliers did not differ markedly from the rest of the population with respect to the characteristics evaluated. When stratified by the median of metabolites fraction, significant associations between urinary arsenic and telomere length were only found in the group with a high fraction of iAs ($\beta = 1.0 \times 10^{-4}$, 95% CI 0.21×10^{-4}–1.8×10^{-4}) and group with a high fraction of MMA ($\beta = 0.88 \times 10^{-4}$, 95% CI 0.12×10^{-4}–1.6×10^{-4}; Table 2) than those below the median ($\beta = 0.13 \times 10^{-4}$, 95% CI -0.85×10^{-4}–1.1×10^{-4} and $\beta = 0.41 \times 10^{-4}$, 95% CI -0.64×10^{-4}–1.5×10^{-4}). All analyses were adjusted for age and BMI.

Table 2. Associations of Urinary Arsenic Concentrations (Sum of Arsenic Metabolites; μg/L) or Fractions (%) of the Different Arsenic Metabolites in Urine and Telomere Length by Linear Regression Analysis

		relative telomere length		
		β-coefficient	95% CI	p value
urinary arsenic[a]				
		0.65×10^{-4}	0.031×10^{-4}–1.3×10^{-4}	0.040
urinary arsenic				
iAs %[b]	low	0.13×10^{-4}	-0.85×10^{-4}–1.1×10^{-4}	0.80
	high	1.0×10^{-4}	0.21×10^{-4}–1.8×10^{-4}	0.014
MMA %[b]	low	0.41×10^{-4}	-0.64×10^{-4}–1.4×10^{-4}	0.44
	high	0.88×10^{-4}	0.12×10^{-4}–1.6×10^{-4}	0.024
DMA %[b]	low	0.63×10^{-4}	-0.13×10^{-4}–1.4×10^{-4}	0.10
	high	0.29×10^{-4}	-0.78×10^{-4}–1.4×10^{-4}	0.59

[a]Linear regression analysis of urinary arsenic (μg/L) vs telomere length, adjusted for age and BMI. [b]Linear regression analysis of urinary arsenic vs telomere length, stratified for the median of fractions of iAs, MMA, or DMA. The analyses were adjusted for age and BMI.

Genetic modification by the two major haplotypes of *AS3MT* was evaluated for the association between urinary arsenic and telomere length. Interactions were found both for the slow and the more toxic-metabolizing *AS3MT* haplotype 1 ($p = 0.036$) and the fast and less toxic-metabolizing haplotype 2 ($p = 0.025$) with arsenic on telomere length. When the analysis of urinary arsenic versus telomere length was stratified for *AS3MT* haplotypes, there was a trend for longer telomere length by arsenic exposure with increasing copy number of haplotype 1 and a trend for longer telomere length for decreasing copy number of haplotype 2 (Table 3). However, these associations were not significant in the groups with two copies of haplotype 1 or haplotype 2.

Urinary arsenic was analyzed in relation to expression of *TERT*, since our main hypothesis was that arsenic affects the telomeres by regulation of expression of this gene. One *TERT* transcript (encodes the longer isoform, isoform 1) was positively associated with arsenic in urine (in the unadjusted analyses, $p = 0.037$, after FDR adjustments $p = 0.38$; Table 4) but not with the telomere length. Urinary arsenic was further evaluated in relation to other telomere-related genes and showed a weak positive association with *RAP1B*. The telomere length was positively associated with expression of three genes [SCY1-like 1 (*SCYL1*; Figure 3), replication protein A1 (*RPA1*), and RAD1 homologue (*RAD1*)], while the telomere length was inversely associated with expression of eight genes [MUS81 endonuclease homologue (*MUS81*), RAP1 interacting factor homologue (*RIF1*), HUS1 checkpoint homologue (*HUS1*), SCY1-like 3 (*SCYL3*), cyclin-dependent kinase 2 (*CDK2*), RAP1B, member of RAS oncogene family (*RAP1B*), golgin, RAB6-interacting (*GORAB*), and topoisomerase (DNA) II α (*TOP2A*)]. After FDR adjustments, none of these associations remained significant.

DISCUSSION

The study showed for the first time that arsenic exposure through drinking water is associated with longer telomeres in vivo. The mechanism for the action of arsenic on the telomere length may probably mediated through up-regulation of telomerase (*TERT*), which was positively associated with arsenic in urine. It is well-known that cancer cells overcome mortality and extend their life span[2] by maintenance of the telomere length, which is often achieved by up-regulation of telomerase.[4] The findings of this study suggest that part of the carcinogenicity of arsenic may be through extending the life span of premalignant cells by elongation of the telomeres. Additionally, the arsenic metabolism efficiency markedly modified the effect of arsenic on telomere length, and individuals carrying the *AS3MT* haplotype associated with a slower and more toxic metabolism of arsenic had longer telomeres as compared to individuals that lack this haplotype. Thus, individuals with a slower arsenic metabolism are probably at increased risk for effects of arsenic on the telomeres and possibly for arsenic-related carcinogenesis.

Table 3. Association (β) between Arsenic Concentrations in Urine and Telomere Length, Stratified for the Number of *AS3MT* Haplotype Copies (Linear Regression Analysis Adjusted for Age and BMI)

haplotype[a]	N copies	N ind.	β	95% CI	p value
haplotype 1	0	92	0.54×10^{-4}	-0.43×10^{-4}–1.5×10^{-4}	0.27
AAGGTTGT	1	58	1.6×10^{-4}	0.52×10^{-4}–2.7×10^{-4}	0.004
	2	16	4.8×10^{-4}	-1.3×10^{-4}–0.0010	0.12
haplotype 2	0	18	4.9×10^{-4}	0.44×10^{-4}–9.4×10^{-4}	0.034
GCCATCAC	1	67	1.1×10^{-4}	0.19×10^{-4}–2.1×10^{-4}	0.019
	2	81	0.69×10^{-4}	-0.45×10^{-4}–1.8×10^{-4}	0.23

[a]Two major *AS3MT* haplotypes are present in this population: (1) AAGGTTGT (associated with a high fraction of MMA and a low fraction of DMA, that is, a slower and more toxic metabolism and (2) GCCATCAC (i.e., low % MMA and high DMA). The order of the polymorphisms in the haplotypes is according to the 5′ to 3′ direction of *AS3MT*: rs7085104, rs3740400, rs3740393, rs3740390, rs11191439, rs11191453, rs10748835, and rs1046778.

Table 4. Correlations (Spearman, r_s) between Gene Expression and Telomere Length as Well as between Gene Expression and Total Concentrations of Urinary Arsenic Metabolites[a]

		telomere			urinary arsenic		
gene[b]	transcript	r_s	*P* value, unadjusted	*P* value, FDR adjusted	r_s	*P*	*P* value, FDR adjusted
SCYL1	ILMN_2400874	0.35	0.001	0.11	0.03	0.80	0.95
SCYL1	ILMN_1731991	0.32	0.002	0.11	−0.05	0.62	0.92
RPA1	ILMN_1795719	0.29	0.007	0.17	−0.06	0.59	0.91
RAD1	ILMN_1763765	0.23	0.036	0.43	0.04	0.71	0.95
MUS81	ILMN_1780937	−0.32	0.003	0.11	−0.10	0.37	0.74
RIF1	ILMN_2149053	−0.31	0.004	0.11	0.01	0.95	0.97
HUS1	ILMN_2136615	−0.26	0.017	0.35	−0.12	0.28	0.68
SCYL3	ILMN_1743427	−0.25	0.021	0.36	0.11	0.29	0.68
CDK2	ILMN_1665559	−0.23	0.031	0.43	−0.01	0.95	0.97
RAP1B	ILMN_1701434	−0.23	0.033	0.43	0.20	0.065	0.41
GORAB	ILMN_2121316	−0.22	0.043	0.47	0.08	0.47	0.83
TOP2A	ILMN_1686097	−0.21	0.050	0.50	−0.03	0.81	0.95
TERT	ILMN_2373119	0.06	0.61	0.92	0.22	0.037	0.38
TERT	ILMN_1796005	−0.12	0.26	0.92	−0.07	0.53	0.88

[a]Note: The ILMN_2373119 was a component of the *TERT* transcript NM_198253.2, which represents the longer transcript and encodes the longer isoform (isoform 1), while ILMN_1796005 was a component of the *TERT* transcript NM_001193376.1, which lacks an alternate in-frame exon in the middle portion of the coding region as compared to NM_198253.2. This results in a shorter protein (isoform 2) as compared to isoform 1 (ref: www.ncbi/nih/gov/gene). [b]Abbreviations: FDR, false discovery rate; *SCYL1*, SCY1-like 1; *RPA1*, replication protein A1; *RAD1*, RAD1 homologue; *MUS81*, replication protein A2; *RIF1*, RAP1 interacting factor homologue; *HUS1*, HUS1 checkpoint homologue; *SCYL3*, SCY1-like 3; *CDK2*, cyclin-dependent kinase 2; *RAP1B*, RAP1B, member of RAS oncogene family; *GORAB*, golgin, RAB6-interacting; *TOP2A*, topoisomerase (DNA) II α; and *TERT*, telomerase reverse transcriptase.

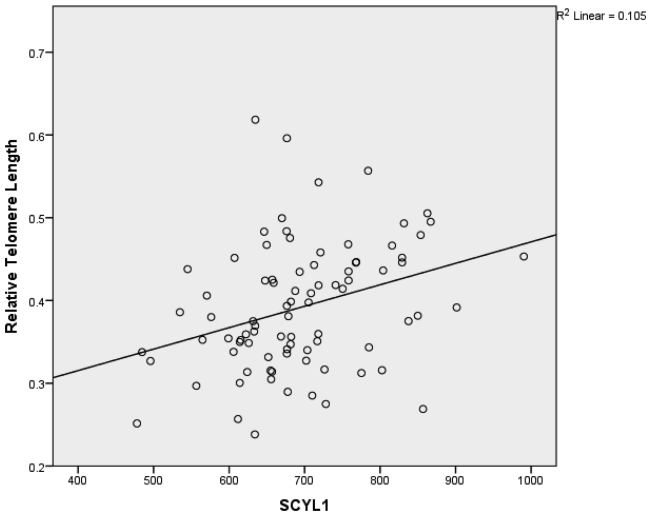

Figure 3. Scatter plot depicting the relationship between the gene expression of *SCYL1* (fluorescence intensity) and the relative telomere length (arbitrary unit) in blood.

There is some support for our results from in vitro and in vivo studies. Arsenite treatment in vitro of keratinocytes or cord blood lymphocytes induced telomerase and telomere length and was also associated with increased cell viability and cell proliferation.[18−20] In a population in Inner Mongolia who were exposed to arsenic via drinking water, an association between expression of *TERT* and arsenic exposure was found, as well as an association between expression of *TERT* and the severity of arsenic-related hyperkeratosis.[19] We found similar arsenic-related increase in *TERT* expression as in the study from Inner Mongolia, and we could also show longer telomeres in relation to arsenic exposure. However, none of the Argentinean women demonstrated hyperkeratosis, whereas in the study from Mongolia, 30% of the individuals had signs of hyperkeratosis. Actually, among the approximately 400 women from this area examined for hyperkeratosis,[26,30] we have not identified one single individual with thickening of the skin. This partly may be explained by the high frequency of the fast arsenic metabolizing haplotype of *AS3MT* in this area, as the *AS3MT* haplotype modified the effect of arsenic on the telomere length.

The expression of telomerase (*TERT*) was positively associated with arsenic in urine, but there was no association between telomere length and *TERT*, which may reflect that these biomarkers are different in time perspective: the effect on telomere length is probably a chronic effect, whereas *TERT* expression reflects a short-term effect of arsenic. The lack of association may also indicate that other processes than telomerase activity are involved in the arsenic-related telomere lengthening. We therefore analyzed expression of genes that influence the telomere stability in vitro in relation to arsenic exposure.

We did not find any evidence that arsenic interacted with their expression, but some of the genes were correlated to telomere length per se, although none of them were significant after FDR adjustments and needs to be cautiously interpreted. Nevertheless, our data indicate a possible role of some telomere-related genes for telomere stability in vivo, and we comment on some of the findings. To our knowledge, this is the first time expression of these genes has been analyzed in relation to human telomere length in vivo. *SCYL1* encodes a protein that can bind specific DNA sequences and can activate transcription of *TERT*, and SCYL1 is involved in DNA damage response when telomeres are shortened in vitro.[36] The positive correlation between *SCYL1* expression and telomere length could indicate that this protein also is up-regulated in vivo and promote longer telomeres or stabilizes the telomere structure. Also, *RAD1* expression, which has a critical role in maintaining telomeres in human cells,[13] was found to be positively associated with telomere length. *RAD1* encodes a component of a heterotrimeric cell cycle checkpoint complex (also known

as the 9−1−1 complex), which localizes to sites of genome damage and promotes DNA damage response signaling.[13,37] Another component of this complex, *HUS1*, was associated with telomere length but in the opposite direction. Telomerase-negative cancer cells can maintain their telomeres via the alternative lengthening of telomeres (ALT) pathway.[38] *MUS81* is an endonuclease that plays a key role in the maintenance of telomeres in cells proficient for ALT,[39] and depletion of *MUS81* in vitro leads to reduced telomere recombination and with growth arrest. *RIF1* encodes a protein that shares homology with the telomere binding protein, Rap1 interacting factor 1, in yeast. *RIF1* is involved in intra-S-phase checkpoint[40,41] and is considered as a negative regulator of telomere length, supported by the inverse correlation effect seen in our study.

Telomere length was inversely associated with age, which has been shown earlier from studies of ours and others.[32,42,43] Also, an inverse association between BMI and telomere length was shown, as reported by others,[5,44−46] supporting the notion that obesity accelerates human aging. Induction of longer telomeres may reflect a difference in the cell population in blood, with younger cells in arsenic-exposed individuals. It was shown by an in vitro study that the telomere length increased in inflammatory cells during acute inflammation.[47] However, subjects in this study were chronically (98% of them had lived in their current village for 2 years or more) exposed to arsenic in drinking water, and arsenic-related elongation of telomeres probably is not reflecting a short-term response.

In conclusion, this study found that urinary arsenic is associated with longer telomeres, in particular in individuals with a genotype associated with a slow arsenic metabolism. The effect of arsenic on telomeres may be through up-regulation of *TERT*, and as well by alteration of other genes that stabilize the telomere structure. Future studies are needed to follow-up if the arsenic-related telomere elongation is associated with increased cancer risk.

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Notes

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ABBREVIATIONS

AS3MT, arsenic (+3 oxidation state) methyltransferase; BMI, body mass index; CI, confidence interval; DMA, dimethylarsinic acid; FDR, false discovery rate; iAs, inorganic arsenic; MMA, methylarsonic acid; SNP, single nucleotide polymorphisms; TERT, telomerase reverse transcriptase

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