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Published in:
Metallomics

DOI:
10.1039/c3mt00365e

2014

Link to publication

Citation for published version (APA):
Rentschler, G., Kippler, M., Axmon, A., Raqib, R., Skerfving, S., Vahter, M., & Broberg Palmgren, K. (2014). Cadmium concentrations in human blood and urine are associated with polymorphisms in zinc transporter genes. *Metallomics*. https://doi.org/10.1039/c3mt00365e

Total number of authors:
7

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Cadmium Concentrations in Human Blood and Urine are Associated with Polymorphisms in Zinc Transporter Genes

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Keywords: ferritin, *SLC39A14*, *SLC39A8*, transporter, zinc, ZIP.

Abbreviations:

B-Cd	Blood cadmium concentration
Cd	Cadmium
Ery-Cd	Erythrocyte cadmium concentration
MAF	Minor allele frequency
LD	Linkage disequilibrium
P-Zn	Plasma zinc concentration
rs	Reference SNP ID
SLC39A8	Solute carrier family 39 (zinc transporter), member 8
SLC39A14	Solute carrier family 39 (zinc transporter), member 14
SNP	Single nucleotide polymorphism
U-Cd	Urinary cadmium concentration
Zn	Zinc

Abstract

Background: Variation in susceptibility to cadmium (Cd) toxicity may partly be due to differences in Cd toxicokinetics. Experimental studies indicate that zinc (Zn) homeostasis proteins transport Cd.

Objective: To evaluate the potential role of variation in Zn-transporter genes (*SLC39A8, SLC39A14*) in Cd concentrations in blood and urine.

Methods: We studied women from the Argentinean Andes [median urinary Cd concentration (U-Cd)=0.24 µg/L; erythrocyte Cd (Ery-Cd)=0.75 µg/L (n=172)] and from rural Bangladesh [U-Cd=0.54 µg/L (n=359), Ery-Cd=1.1 µg/L (n=400)]. Polymorphisms (n=36) were genotyped with Sequenom. Gene expression was measured in whole blood with Illumina DirectHyb HumanHT-12 v4.0.

Results: Polymorphisms in *SLC39A8* and *SLC39A14* were associated with Ery-Cd concentrations in the Andean population. For *SLC39A14,* women carrying GT or TT genotypes of rs4872479 had 1.25 (95% confidence interval (CI)=1.07-1.46) times higher Ery-Cd than women carrying GG. Also, women carrying AG or AA of rs870215 had 1.17 (CI 1.01-1.32) times higher Ery-Cd than those carrying GG. For *SLC39A8,* women carrying AG or GG of rs10014145 had 1.18 (CI 1.03-1.35) times higher Ery-Cd than those with AA, and carriers of CA or AA of rs233804 showed 1.22 (CI 1.04-1.42) times higher Ery-Cd than CC. The Bangladeshi population had similar, but statistically non-significant associations between some of these SNPs and Ery-Cd. In the Andean population, the genotypes of *SLC39A14* rs4872479 and rs870215 associated with lower Ery-Cd, showed positive correlations with plasma-Zn (P-Zn) and *SLC39A14* expression.

Conclusions: Polymorphisms in *SLC39A14* and *SLC39A8* seemed to affect blood Cd concentrations, for *SLC39A14* this effect may occur via differential gene expression.

Introduction

The widespread, toxic element cadmium (Cd) adversely affects kidneys and bone, even at the low exposure levels found in the general population world-wide[1-4]. Cd exposure occurs mainly from smoking and from food, such as cereals, vegetables and shellfish[5]. The intestine absorbs very little Cd, usually less than 5% of the amount ingested[6], but this varies widely. In particular, individuals with low iron stores absorb more Cd[7-9]. Cd accumulates in the kidney; therefore, even a small increase in absorption rate could have severe effects later in life.

Twin-studies showed that genetic factors influence Cd kinetics in humans[10-11], and women showed a more pronounced genetic effect than men[10]. One study of 370 human volunteers from Thailand found an association of the glutathione S-transferase polymorphism *GSTP1* rs1695 and Cd concentrations in blood[12]. Furthermore, a polymorphism in the metallothionein IIA gene *MT2A* was associated with differences in Cd concentrations in human kidney cortex[13]. We recently showed that one polymorphism in the iron-related transferrin receptor gene *TFRC* was associated with differences in Cd concentrations in women´s urine, a marker of long-term Cd accumulation in the kidney[14].

Cd interacts with zinc (Zn) and binds with high affinity to Zn-binding metallothioneins and Zn-finger proteins[15]. Also, Cd may interact with the Zn transporters encoded by *SLC39A8* and *SLC39A14*, as shown *in vitro* and in animal experiments[16-18]. *SLC39A8* and *SLC39A14* are expressed in the intestine and in the kidney, but the role of the encoded proteins in Cd toxicokinetics *in vivo* remains unclear.

This study aimed to elucidate whether polymorphisms in *SLC39A8* and *SLC39A14*, belonging to the Zn-homeostasis system, modify Cd concentrations in blood and urine. We studied this in two different populations, one in the Argentinean Andes and one in Bangladesh.

Results

Characteristics of study participants

Compared with the Andean women, the Bangladeshi women had higher Ery-Cd and U-Cd, but lower BMI, P-Zn and plasma ferritin (Table 1). Using 0.5 mg/L as the threshold for adequate/low Zn concentrations[19], only 6% of the Andean women, but 35% of the Bangladeshi women had low Zn concentrations. Ery-Cd and U-Cd were positively correlated with age in both populations (all p-values <0.001; Supplementary Material, Table 4). In the Andean women, P-Zn correlated weakly with U-Cd (r_S=0.20; p<0.05), age (r_S=0.24; p<0.05), and parity (r_S=0.25; p<0.05), but not with Ery-Cd. In the Bangladeshi women, P-Zn did not correlate with any of those, but Ery-Cd correlated inversely with ferritin (r_S=-0.18; p<0.001).

For the SNPs rs10014145 and rs233804 (*SLC39A8*), minor allele frequencies (MAFs) differed more than 20% between the populations (Table 2, Supplementary Material, Table 1), whereas MAFs of rs4872479 and rs870215 (*SLC39A14*) did not differ substantially (<6%). The SNPs rs10014145, and rs233804 (*SLC39A8*) were not in linkage disequilibrium (LD) in either population (r^2=11 and 10%, respectively). The SNPs rs4872479, and rs870215 in *SLC39A14* were in weak LD in the Andean (r^2=66%) but not in the Bangladeshi populations (r^2=15%).

Associations of polymorphisms with differences in metal biomarkers

SLC39A14

In models adjusted for age only, women with the GT or TT genotypes of rs4872479 showed 1.25 (95% confidence interval [CI] 1.07-1.46) times higher Ery-Cd than women with GG in the Andean, but not in Bangladeshi (1.06 [CI 0.92-1.23]; Table 2) populations. Women with GT or TT also showed non-significantly higher U-Cd in both populations (1.21 and 1.17,

respectively). Carriers of the AG or AA genotypes of rs870215 had 1.17 (CI 1.01-1.32) times higher Ery-Cd and 1.23 (CI 0.96-1.57) times higher U-Cd in the Andean population, compared to GG carriers. In the Bangladeshi population, we detected a trend of increased Ery-Cd in women with AA (1.12 [CI 0.82-1.58] times higher) and AG genotypes (1.07 [CI 0.96-1.20] times higher) compared with those with GG genotypes (p-value for trend = 0.18). Additionally adjusting the Cd models for P-Zn, parity or BMI did not change the results substantially; i.e., the level of statistical significance remained unaffected and the effect estimates changed by less than ±0.05. Also, adjusting for ferritin affected the estimates very little (less than ±0.07), except that the association between rs870215 and Ery-Cd became statistically non-significant 1.12 (CI 0.97-1.29).

The SNPs were not statistically significantly associated with differences in P-Zn (Table 2). However, carriers of GT/TT of rs4872479 showed lower ferritin concentrations in both populations, and the difference was statistically significant in the Andean women (Table 2). Homozygote but not heterozygote carriers of rs870215 had higher P-ferritin in the Bangladeshi women. One non-synonymous (rs896378, P33L) and one synonymous (rs2293144, L65L) SNP were also analyzed. The MAF was sufficient for both SNPs to allow us to calculate associations with differences in Ery-Cd and U-Cd. However, we detected no associations (data not shown).

SLC39A8

The AG or GG carriers of rs10014145 in the Andean women showed 1.18 (CI 1.03 – 1.35) times higher Ery-Cd, and 1.23 times higher U-Cd (CI 0.97-1.54) compared with AA (Table 2). In the Bangladeshi women, rs10014145 GG carriers showed 1.15 (CI 0.99-1.33; p for trend =0.04) higher Ery-Cd but not higher U-Cd. The CA or AA carriers of rs233804 in the Andean women showed 1.22 (CI 1.04-1.42) times higher Ery-Cd and 1.34 (CI 1.03-1.76)

times higher U-Cd (Table 2). Heterozygote carriers for rs233804 were associated with Ery-Cd in the Bangladeshi population (Table 2). Adjusting the models for P-Zn, parity or BMI did not change the results substantially; the level of significance remained unaffected and the effect estimates changed by less than ±0.02. Also, adjusting for ferritin minimally altered the effect estimates (less than ±0.05).

One non-synonymous (rs13107325, A391T) and one synonymous (rs17823966, H347H) SNP were evaluated for association with differences in Ery-Cd or U-Cd; however, the MAF was too low for rs13107325 and for rs17823966 we found no associations (not in table).

The other SNPs of *SLC39A8* or *SLC3914* (Supplementary Table 1) did not show any statistically significant associations with differences in Ery-Cd, U-Cd or P-Zn.

Associations between gene expression and biomarkers of cadmium, zinc and iron

To determine whether these SNPs affected gene expression, we measured *SLC39A8* and *SLC39A14* expression in peripheral blood. CA or AA carriers of rs233804 had statistically significantly lower expression of *SLC39A8* for one of the expression probes (Supplementary Tables 3 and 5). There was no clear genotype-specific association with expression of *SLC39A14*.

In the Andean women, the expression of *SLC39A14* was positively correlated with P-Zn (Table 3A). When the Andean group was split by rs4872479 genotype, the GG carriers showed a statistically significant positive correlation of *SLC39A14* expression with P-Zn, but there was no correlation for GT (Table 3B). However, for GT carriers, U-Cd was inversely associated with *SLC39A14* expression. A similar pattern was observed when the group was split by rs870215: for GG carriers, *SLC39A14* expression was correlated with P-Zn, while for AG the expression was inversely associated with U-Cd.

Expression of *SLC39A8* was not correlated with zinc or Cd biomarkers (Supplementary Table 6).

Discussion

In the present study we found evidence that intronic variants of the Zn-transporter genes *SLC39A14* (rs4872479 and rs870215) and *SLC39A8* (rs10015145 and rs233804) are associated with the kinetics of Cd. The findings of higher Cd concentrations among carriers of the rare variants appear to be consistent between the two population groups. The associations with S*LC39A14* and *SLC39A8* and Cd, if confirmed in other populations, could be important to explain variation in Cd toxicity in bone and kidney, in particular in populations where alleles related to high Cd concentrations are common. *SLC39A14* rs870215 is present in 40-67 % of African populations analysed[20] and *SLC39A8* rs10014145 in 36% of Bangladeshis, 33-36% in Europeans and 31-40% in Africans. There were no evident effects of the studied SNPs on P-Zn concentrations; however, in the Andean group we found genotype-specific associations for gene expression of *SLC39A14* (rs4871479, rs870215) and P-Zn, suggesting a role of these SNPs in zinc status.

The associations were stronger in the women from the Argentinean Andes than in the pregnant women from Bangladesh, although the blood Cd concentrations were higher in the latter group. Possibly, the lower zinc and iron status in the Bangladeshi women, as judged by the P-Zn and ferritin concentrations, played a role, but we could not find any clear evidence for that. The Bangladeshi women were also leaner that those in the Andes, but BMI did not modify the associations between genotypes and differences in Cd concentrations, indicating that other nutritional factors did not explain the differences in strength of associations between the study groups. In summary, the differences between the populations (BMI, age, parity, P-Zn and P-ferritin) were mathematically adjusted and did only play a minor role for

the associations between SNPs and differences in Cd concentrations. The advantages with comparing these two different populations were (1) each group was homogenous (2) they represented different levels of Cd exposure with a wide distribution (3) there were no other sources of Cd exposure (e.g. industrial pollution or smoking).

Both populations have a well-known exposure to other metals, mainly arsenic[21-22]. Therefore we have adjusted the statistical models for total urinary arsenic and found that it did not contribute to the associations between SNPs and differences in Cd concentrations. Besides, arsenic metabolism is strongly associated with a very different set of genes, one of which is *AS3MT*[23]. Therefore the exposure to As did not hinder studying associations of *SLC39A8* and *SLC39A14* with Cd toxicokinetics.

The fact that the populations live at different altitudes could have played a role. It has been suggested that the genetic factors regulating the metabolism of zinc and iron, both of which are essential for heme synthesis and thus protective against hypoxia, account for a larger fraction of the elements concentrations in the body in populations residing at high compared with low altitude[24-25]. Still, we did not observe a stronger genetic effect on zinc or iron status in Argentina compared to Bangladesh. It should be mentioned though that *SLC39A14* was associated with differences in ferritin concentrations in both populations, a finding that might reflect that *SLC39A14* is involved in transport of iron in its non-transferrin bound form to the liver[26].

We found stronger associations between SNPs and differences in Cd in blood than in urine. Zinc metabolism has a faster turnover compared to other nutrients because stored pools are very small[27]. Therefore it seems logical that variation in Cd biomarkers in connection to Zn metabolism would be more evident in the short term (B-Cd) than in the long-term (U-Cd) marker. The use of U-Cd concentrations as marker of kidney damage at low exposure levels is limited by the inter-individual variation in tubular uptake[28]. *SLC39A8* and *SLC39A14* are

ingoing transporters; therefore their increased expression in kidney proximal tubuli would lead to increased re-uptake of Cd and consequently reduced release of Cd into urine. Thus, small changes in re-absorption could conceal increased Cd accumulation in the kidneys for many years before the toxic effects will become apparent.

Some observations were made using gene expression data that was available for a sub-group of the Andean population. The positive correlation between expression of *SLC39A14* and P-Zn is in accordance with earlier animal studies[29-30]. We also found indications that this association was specific for GG carriers of rs4871479 or rs870215. Zn signaling or inflammation increases the expression of *SLC39A14,* resulting in increased Zn absorption of in the gastrointestinal tract and increased Zn transfer into cells, e.g. in the liver[29, 31]. Compared to liver, the expression level of *SLC39A14* in blood is low[32] and the relation between gene expression in blood in relation to other tissues needs to be further investigated before firm conclusions can be made. However, we speculate that for *SLC39A14* rs4872479, the G variant is expressed in response to a need for extra Zn, but not the T variant as it abolishes a binding site for transcription factors of the CEBP family (regulating DNA repair, immune response and wound repair). The same effect of rs4871479 or rs870215 on expression might reflect the LD between them. As *SLC39A14* is an ingoing transporter, expression in kidney proximal tubuli will also contribute to increase P-Zn but will at the same time lead to decreased U-Cd. We observed no association between *SLC39A8* expression and Cd or P-Zn, maybe because we studied blood with low expression of *SLC39A8*[16, 31] and no further conclusions can be drawn from our data regarding the mechanisms of action of the SNPs in this gene.

Conclusion

This study suggests that genetic variation in Zn transporters *SLC39A8* and *SLC39A14* influences Cd concentrations in human blood, meaning that individuals with a certain

genotype of these transporters have higher Cd concentrations. Genotype-specific gene expression seems to be the mechanism underlying the effect of *SLC39A14* SNPs on Cd kinetics. If the same associations between genotype and differences in Cd concentrations can be found in other populations, these results explain part of the variation in Cd kinetics and toxicity.

Experimental

Study areas and populations

For this study we used samples and Cd data collected in two previous cross-sectional studies, involving two different populations, one in the Argentinean Andes[33] and one in rural Bangladesh[19]. The Health Ministry of Salta, Argentina, the Ethical Review Committee of icddr,b, and the Regional Ethics Committee in Stockholm, Sweden, approved the study. Oral and written informed consents were obtained from all participants.

Argentinean Andes

The women included were part of a cross-sectional study on health effects of elements in drinking water and food in San Antonio de los Cobres and surrounding villages on the Andean plateau (about 4000 m above sea level) in Northwestern Argentina (Table 1). The sampling of blood, urine, and plasma was performed in 2008[33]. Among the first-degree relatives, we excluded 31 daughters to included mothers; thus, 172 female volunteers remained. Only three of the women smoked tobacco, the water concentrations of Cd were low (<0.17 µg/L), and this area has no known industrial Cd pollution; therefore, the main source of Cd exposure was the food[34]. A sub-group of 72 non first-degree relatives were chosen for gene expression analysis among samples that had RNA integrity better than 7.5 (randomly chosen for Cd and Zn biomarker levels). The listed characteristics of the Andean sub-group

analysed for gene expression did not differ significantly from the whole population (Table 1), except that they were slightly younger (median 34 vs. 36 years).

Bangladesh

The studied women (Table 1) were part of a longitudinal study on health effects of early-life exposure to environmental pollutants in Matlab, a rural area 53 km southeast of Dhaka[19, 35]. The women were non-smokers living in an area with no known sources of industrial Cd pollution; thus, as in the Andean population, the Cd exposure was mainly via food[36]. Samples were obtained in early pregnancy, urine at gestational week 8 (range 4-19) and blood at gestational week 14 (range 9-22)[19]. DNA samples from 403 women were available. Among those, measurements of cadmium in both urine and blood were available for 359 and in blood only for an additional 41 women.

Metal biomarkers

Cadmium

Cadmium concentration in urine (U-Cd) is an index of the body burden, particularly the concentrations in the kidneys, whereas Cd in blood (B-Cd) mainly reflects on-going exposure, with some influence from the body burden[4, 37]. Erythrocyte-Cd (Ery-Cd; Bangladeshi samples), B-Cd (Argentinean samples) and U-Cd were measured using inductively coupled plasma mass spectrometry (ICPMS; Agilent 7500ce, Agilent Technologies, Tokyo, Japan), with the collision cell in helium mode to avoid interference from molybdenum, as described in detail elsewhere[14, 19, 34]. Limit of detection (LOD) for B-Cd was 0.011 µg/L for samples from the Argentinean Andes and <0.1 µg/L for Bangladeshi erythrocyte samples. LOD for U-Cd was <0.05 µg/L in both populations. All samples contained Cd concentrations above the different LODs. Accuracy was ascertained by reference materials for which the obtained Cd concentrations showed a good agreement with certified or recommended concentrations[9, 19].

To compare the blood Cd concentrations in the two study groups, the whole B-Cd from the Andean group was recalculated to Ery-Cd assuming that 95% of the Cd is bound in erythrocytes[37] and that the density of our erythrocyte preparations was 1.055 g/mL. To account for the volume fractions of erythrocytes and plasma, we used the measured hemoglobin concentration of each woman, divided by 340 g/L[38-39]. The median and range of hemoglobin in this group was 156 (90-202) g/L.

To compensate for variations in the dilution of urine, concentrations were adjusted to the mean specific gravity for each population (1.020 g/mL in Argentina and 1.012 g/mL in Bangladesh), measured by a digital refractometer (EUROMEXRD712 clinical refractometer; EROMEX, Arnhem, Holland). Because of major differences in body size and meat intake it was not possible to compare creatinine-adjusted urine concentrations between the two groups of women.

Plasma zinc and plasma ferritin

Plasma Zn concentrations (P-Zn) in the Andean women were measured by spectrophotometry (accredited method, LOD 0.6 µmol/L, imprecision <2.7 %). P-Zn in Bangladeshi women was assessed by atomic absorption spectrophotometry (imprecision <2.0 %)[40]. Ferritin concentrations were analysed by immunoassay (Andean samples) and radioimmunoassay (Bangladeshi samples) as previously reported[14, 19].

Genetic analyses

Genotyping of single nucleotide polymorphisms

Only a few SNPs in *SLC39A8* and *SLC39A14* have been shown to affect gene expression or protein function. Moreover, many of the SNPs that alter the coding sequence or may lead to a truncated protein are very rare and, thus, would not be expected to explain a major part of the

variation in Cd kinetics. Therefore, we used another strategy and selected SNPs (most of them with no functional information) from those that can tag genetic variation in a larger part of the gene by linkage to other SNPs (so called tagSNPs). TagSNPs were calculated with Haploview (version 4.1) using data from Hapmap for *SLC39A8* and *SLC39A14*. The threshold of minor allele frequency (MAF) was 5% based on the MAFs in the Asian Hapmap populations (Han Chinese in Beijing, China and Japanese in Tokyo, Japan), the populations closest to the Bangladeshi at the time of selection of the SNPs. Despite low the MAF, we included one synonymous and one non-synonymous SNP from each of the two genes according to dbSNP[20].

DNA was isolated from peripheral blood by the QIAmp DNA Blood Mini kit (QIAGEN, Hilden, Germany) by Swegene´s DNA facility at Malmö University Hospital, Malmö, Sweden. Altogether, 39 SNPs were genotyped using Sequenom (San Diego, CA, USA) technology by Swegene´s DNA facility at Malmö University Hospital, Malmö, Sweden.

The quality control was as follows: if the call algorithm automatically defined the genotype in at least 90% of the samples, the quality of the assay was sufficient and the SNP was accepted; if the call algorithm automatically reported a genotype for more than 60% of the SNPs, the DNA quality was sufficient for a sample to be accepted. This resulted in exclusion of 3 SNPs, while the DNA quality was sufficient for all samples to be included. The final data analysis was thus based on 36 SNPs (Supplementary Material, Table 1).

Bioinformatics using the ElDorado database (version 08-2011) was performed to identify transcription-factor sites that may be affected by SNPs (http://www.genomatix.de/en/index.html; Supplementary Material, Table 2).

RNA collection and gene expression analysis

In a subgroup of 122 women from the Andes (the first women sampled), peripheral blood was collected in PAX tubes (PreAnalytiX GmbH, Hombrechtikon, Switzerland). RNA was extracted and checked for quality as described in detail elsewhere[33]. For the gene expression analysis, 72 non first-degree relatives were chosen among samples that had RNA integrity better than 7.5 (randomly chosen for Cd and Zn biomarker levels). For the gene expression analysis, DirectHyb HumanHT-12 v4.0 (Illumina, San Diego, CA, USA) was used, according to the manufacturer's instructions, and the analysis was performed at the SCIBLU laboratory at Lund University. Probes for the gene expression analysis are listed in Supplementary Material, Table 3. Filtering of the gene expression data was done by BioArray Software Environment (BASE) in an intensity-dependent manner. Results are presented as relative fluorescence units.

Statistical analysis

The study groups from the Argentinean Andes and Bangladesh were analysed separately. We tested deviations from Hardy-Weinberg equilibrium by chi-square analysis. Linkage disequilibrium (LD) analysis was performed with Haploview[41]. Among those SNPs that were in LD ($r^2 > 80\%$), one was chosen randomly to represent linked groups.

Associations of genotypes with differences in metal concentrations (dependent variables) were analysed using multivariable-adjusted linear regression analyses. Initially, all models for Cd were adjusted for age since age was correlated with the Cd biomarkers. Thereafter, we additionally adjusted the Cd models for P-Zn, plasma ferritin, parity and BMI. Associations for genotypes with differences in ferritin and zinc were tested in unadjusted models. To obtain normally distributed residuals, U- Cd and Ery-Cd were naturally log (ln) transformed. We present the relative changes (%) of metal concentrations for a variant genotype compared to the most common homozygote genotype in the largest study population, i.e. the one from the Bangladeshi (reference) population, making it possible to

compare the effect in two different populations despite their differences in metal concentrations. In general, each polymorphism was analysed as three genotypes, except when the frequency of a homozygote genotype was too low (<8 individuals); then this group was pooled with the heterozygotes. In total, 19 independent tests were performed for associations between SNPs and differences in metal concentrations (Ery-Cd, U-Cd and P-Zn). We used the false discovery rate (FDR) procedure to adjust for multiple comparisons [R version 2.14.2 (http:// www.r-project.org/)] in the Andean group where we had statistically significant findings.

Correlations between metal concentrations and gene expression were made using the Spearman correlation coefficient (r_s). Relations between SNPs and gene expression data were analysed by Kruskal-Wallis tests.

All calculations were made with SPSS statistics version 20. "Statistical significance" refers to $p<0.05$ (two-tailed).

Acknowledgements

This research was supported by the Swedish Council for Working Life and Social Research (FAS; project “METALUND”), Karolinska Institutet, Kungliga fysiografiska sällskapet, EU (FP6; project “PHIME” FOOD-CT-2006-016253). The Bangladeshi samples were part of the MINIMat study, funded by UNICEF, Swedish International Development Cooperation Agency (SIDA), UK Medical Research Council, Swedish Research Council, Department for International Development (DFID), and International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b).

The authors declare they have no actual or potential competing financial interests.

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Tables

Table 1. General characteristics of the two groups of women.

Variable	Argentinean Andes						Bangladesh		
	All			Sub-group [a]					
	N	Median	Range	N	Median	Range	N	Median	Range
Age[b] (years)	172	36	12-80	72	34	12-65	403	26	14-44
BMI	172	25	16-40	72	24	16-36	400	20	14-29
Parity	167	4	0-14	70	3	0-14	403	1	0-7
Cadmium in blood[b] (µg/L)	172	0.36	0.17-1.0	72	0.32	0.17-1.1	-	-	-
- erythrocytes[b,c] (µg/kg)	172	0.75	0.36-2.1	72	0.68	0.37-1.9	400	1.1	0.31-5.4
- urine[b,d] (µg/L)	172	0.24	0.01-1.5	72	0.22	0.01-1.5	359	0.54	0.05-4.5
Zinc in plasma[b] (mg/L)	157	0.72	0.39-4.8	64	0.72	0.39-4.8	399	0.56	0.27-2.1
Ferritin in plasma[b] (µg/L)	166	52	4-1200	70	48	4-320	399	30	2.6-200

[a] Sub-group included in gene expression analyses.
[b] Figures for Argentinean Andes and U-Cd in Bangladesh were published earlier in Rentschler et al.[14].
[c] Cd in erythrocytes calculated in Argentinean Andes, measured in Bangladesh as described in Methods.
[d] Adjusted for specific gravity.

Table 2. Relative changes in cadmium concentrations in urine (U-Cd) and blood (Ery-Cd) (age-adjusted models), and zinc concentrations in plasma (P-Zn) (unadjusted models) between genotypes of *SLC39A14* and *SLC39A8*. Reference genotype is the most common homozygote in the Bangladeshi women (CI = 95% confidence interval)[a].

Gene SNP	Population	Genotype	N	Ery-Cd (CI)	N	U-Cd (CI)	N	P-Zn (CI)	N	P-Ferritin (CI)
SLC39A14										
rs4872479	Andes	GG	144	**1.0**	144	1.0	131	1.0	139	**1.0**
		GT/TT[b]	24	**1.25 (1.07-1.46)[c]**	24	1.21 (0.92-1.59)	22	1.03 (0.92-1.16)	23	**0.62 (0.38-0.99)**
	Bangladesh	GG	337	1.0	304	1.0	340	1.0	301	1.0
		GT/TT[d]	50	1.06 (0.92-1.23)	42	1.17 (0.91-1.50)	51	0.95 (0.87-1.05)	41	0.90 (0.71-1.14)
rs870215	Andes	GG	136	**1.0**	136	1.0	123	1.0	131	1.0
		AG/AA[e]	32	**1.17 (1.01-1.32)[f]**	32	1.23 (0.96-1.57)	30	1.07 (0.96-1.19)	31	0.88 (0.57-1.35)
	Bangladesh	GG	276	1.0	246	1.0	278	1.0	276	1.0
		AG	104	1.07 (0.96-1.20)	102	1.00 (0.84-1.20)[h]	105	0.96 (0.90-1.03)	103	0.83 (0.71-0.98)
		AA[g]	9	1.12 (0.82-1.58)			9	1.07 (0.87-1.32)	9	**1.68 (1.05-2.71)**
SLC39A8										
rs10014145	Andes	AA	133	**1.0**	133	1.0	121	1.0	128	1.0
		AG/GG[i]	37	**1.18 (1.03–1.35)[j]**	37	1.23 (0.97-1.54)	34	0.91 (0.82-1.00)	36	1.15 (0.76-1.72)
	Bangladesh	AA	169	1.0	148	1.0	170	1.0	145	1.0
		AG	165	1.09 (0.98-1.21)	152	0.98 (0.83-1.17)	165	0.97 (0.91-1.04)	151	0.98 (0.83-1.15)
		GG[k]	58	1.15 (0.99-1.33)	52	0.99 (0.78-1.27)	58	0.99 (0.90-1.08)	52	0.96 (0.76-1.21)
rs233804	Andes	CC	142	**1.0**	142	**1.0**	127	1.0	136	1.0
		CA/AA[l]	25	**1.22 (1.04-1.42)[m]**	25	**1.34 (1.03-1.76)**	25	0.98 (0.88-1.10)	25	1.34 (0.84-2.14)
	Bangladesh	CC	166	1.0	144	1.0	165	1.0	142	1.0
		CA	180	**1.13 (1.01-1.25)**	168	0.99 (0.83-1.18)	181	0.98 (0.91-1.04)	166	0.93 (0.79-1.09)
		AA[n]	54	0.97 (0.84-1.13)	47	1.02 (0.79-1.32)	55	0.92 (0.84-1.02)	47	0.95 (0.75-1.20)

[a] In cases where the frequency of a homozygote genotype was low (<8 individuals), this group was pooled with the heterozygotes.
Minor allele frequencies: [b] 7%; [d] 7%; [e] 10%; [g] 16%; [i] 13%; [k] 36%; [l] 8%; [n] 36%.
[h] P-value for 3 genotypes 0.6; (N_{GG}=246; N_{AG}=95; N_{AA}=7).
False discovery rate (FDR) adjusted p-values: [c] 0.057; [f] 0.08; [j] 0.057; [m] 0.08;

Table 3. Spearman's rank correlations (r_S) between concentrations of cadmium in blood (B-Cd, µg/L), cadmium in urine (U-Cd, µg/L), zinc in plasma (P-Zn, mg/L), or plasma ferritin (µg/L) on the one hand and expression of *SLC39A14* in whole blood on the other. Results are presented in all women (A), and stratified by genotype (B), in the Andean population. N = number of women with samples for gene expression.

A.		B-Cd	U-Cd	P-Zn	Ferritin
Total expression	r_S	-0.11	-0.10	**0.27**	0.03
	P	0.4	0.4	**0.03**	0.8
	N	72	72	64	70
B. Expression by SNP Genotype					
rs4872479					
GG	r_S	-0.09	-0.003	**0.30**	0.08
	P	0.5	1.0	**0.03**	0.6
	N	61	61	54	59
GT	r_S	-0.22	**-0.73**	-0.13	-0.26
	P	0.5	**0.02**	0.8	0.5
	N	10	**10**	9	10
rs870215					
GG	r_S	-0.12	0.03	**0.31**	0.14
	P	0.4	0.8	**0.03**	0.3
	N	58	58	**51**	56
AG	r_S	-0.20	**-0.82**	0.02	-0.43
	P	0.5	**0.0007**	1.0	0.1
	N	13	**13**	12	13

Supplementary material

Cadmium Concentrations in Human Blood and Urine are Associated with Polymorphisms in Zinc Transporter Genes

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Table 1 (supplementary material). Polymorphisms of the *SLC39A14* and *SLC39A8* genes that were successfully genotyped. Quality requirements: HWE[a)] and 90% of samples easily recognized in the mass spectrometry analysis

Gene	rs nr [b]	Polymorphism type [c]	Allele frequencies Argentina	QC (%)[a]	Allele frequencies Bangladesh	QC (%)[a]
SLC39A14	rs870215	Intron A>G	10/90	97	16/84	96
	rs896377	Intron C>G	4/96	97	22/78	97
	rs896378	P33L T>C	48/52	98	43/57	99
	rs1051638	3' UTR G>A	44/56	98	45/55	98
	rs2293144	L65L A>G	40/60	98	37/63	99
	rs4872479	Intron T>G	7/93	97	7/93	96
	rs7818776	Intron A>C	55/45	96	39/61	95
	rs11783730	Intron T>C	48/52	97	33/67	99
	rs11989798	Intron C>A	3/97	96	13/87	95
	rs13259873	Intron T>C	51/49	98	47/53	99
SLC39A8	rs9705	3' UTR A>G	60/40	98	23/77	98
	rs233804	Intron A>C	8/92	97	36/64	100
	rs233818	Intron T>C	63/37	95	27/73	97 (*)
	rs233825	Intron G>A	12/88	97	36/64	98 (*)
	rs233827	Intron A>G	15/85	96	53/47	98
	rs390878	Intron T>C	60/40	96	28/72	98
	rs971752	Intron G>A	16/84	97	26/74	97
	rs998719	Intron C>T	11/89	96	22/78	98
	rs1462947	Intron A>G	-	84(**)	32/68	92
	rs1545744	Intron T>A	16/84	100	25/75	99
	rs2165265	Intron G>T	23/77	93 (*)	20/80	96
	rs4699011	Intron T>C	16/84	98	26/74	99
	rs6822371	Intron T>G	19/81	97	30/70	98
	rs6829701	Intron A>G	10/90	97	31/69	99
	rs6848517	Intron C>G	70/30	97	38/62	100
	rs7436937	Intron T>G	70/30	97	37/63	95
	rs7655493	Intron C>T	27/73	92	37/63	94
	rs7664683	Intron T>C	2/98	97	23/77	99
	rs7681239	Intron C>T	3/97	96	18/82	93
	rs10014145	Intron G>A	13/87	98	36/64	97
	rs11941736	Intron C>G	10/90	98	30/70	98
	rs13103835	Intron G>A	19/81	97	30/70	98
	rs13107325	A391T G>A	1/99	98	1/99	99
	rs17032363	3' UTR G>A	0/100	97	1/99	94
	rs17226148	Intron G>A	7/93	96	13/87	98
	rs17823966	H347H C>T	0/100	100	8/92	99

[a] If there was Hardy-Weinberg disequilibrium in both populations, the polymorphism was excluded. Polymorphisms that demonstrated disequilibrium in one of the populations are marked with (*). A polymorphism was excluded in one population for quality reasons (**).

[b] Rs numbers from NCBI SNP Database (website: http://www.ncbi.nlm.nih.gov/SNP).

[c] When applicable, amino acid position/gene region is denoted.

Table 2 (supplementary material). Modification by SNPs of putative transcription factor binding sites.

Gene SNP	Allele	Effect	Site affected Family/Factor	Description
SLC39A14				
rs4872479	G -> T	lost	CEBP/CEBP02	CCAAT/enhancer binding protein
rs870215	G -> A	new	HOXH/MEIS1A_HOXA	Meis homeobox 1
SLC39A8				
rs10014145	A -> G	lost	NKXH/NKX31	NK3 homeobox 1, NKX-3 ALPHA
rs233804	C -> A	new	TALE/MRG1	Meis homeobox 2 (Meis2)

Table 3 (supplementary material). Expression probes for *SLC39A8 and SLC39A14.*

Gene	Probe No	Sequence	Median[a] (min-max)
SLC39A8	ILMN_1695316	GGTTGCACCCCTCACAAATGGCAGAACAGTATGTAAAGCTGGTAACACCT [b]	155 (110-210)
	ILMN_2233539	GTGTGATCGAGAGCCATTCAGAAAAGACTTCCTTTGTGTTCAGCCTATAC [b]	163 (97-280)
SLC39A14	ILMN_1764629	TGTCACGTGCAGGAACAGTGAGGCAGGGACAGGGGTTCTGCTCCTTCTCA [c]	102 (83-120)

[a] Expression in relative fluorescence units.
[b] Located on exon 8b covering transcripts 1, 2 and 4 but not 3 (www.ncbi.nih.gov/nucleotide).
[c] Located on exon 11 covering transcripts 1, 2 and 3 (www.ncbi.nih.gov/nucleotide).

Table 4 (supplementary material). Spearman's correlation coefficients (r_S) between cadmium levels in erythrocytes (Ery-Cd), blood (B-Cd) and urine (U-Cd), plasma zinc (P-Zn), ferritin in plasma, age and parity in the Bangladeshi and Argentinean Andes populations.

		Parity		Ery-Cd[a]		B-Cd		U-Cd		P-Zn		Plasma Ferritin	
		Andes	Bangladesh	Andes[b]	Bangladesh	Andes[b]	Bangladesh	Andes[b]	Bangladesh	Andes	Bangladesh	Andes[b]	Bangladesh
Age	r_S	0.63	0.76	0.33	0.21	0.40	—	0.42	0.28	0.24	-0.08	0.50	0.03
	P	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001	0.002	0.1	<0.001	0.6
	N	167	403	172	400	172	—	172	340	157	399	166	399
Parity	r_S			0.33	0.19	0.40	—	0.41	0.31	0.25	-0.08	0.30	0.03
	P			<0.001	<0.001	<0.001		<0.001	<0.001	0.002	0.1	<0.001	0.6
	N			167	400	167	—	167	340	154	399	161	399
Ery-Cd	r_S					0.97	—	0.42	0.47	0.03	-0.02	-0.09	-0.18
	P					<0.001		<0.001	<0.001	0.7	0.6	0.3	<0.001
	N					172	—	172	337	157	396	166	396
B-Cd	r_S							0.45	—	0.11	—	0.03	—
	P							<0.001		0.2		0.7	
	N							172	—	157	—	166	—
U-Cd	r_S									0.20	-0.07	0.21	-0.10
	P									0.01	0.2	0.008	0.06
	N									157	336	166	336
P-Zn	r_S											0.18	0.06
	P											0.02	0.3
	N											156	397

[a] Cd in erythrocytes calculated in Argentinean Andes, measured in Bangladesh.
[b] Numbers for Argentinean Andes quoted from EHP, 121 (4) 2013, 467-472.
— Not measured.

Table 5 (supplementary material). Gene expression (relative units) as a function of genotype measured in a subset of the Andean population (P-values of Kruskal-Wallis test).

Gene SNP	Genotype	Median	Range	N	P-value
SLC39A14					
rs4872479	GG	101	83 - 120	61	0.7
	GT	100	92 - 110	10	
rs870215	GG	101	83 - 120	58	0.8
	AG/AA	100	92 - 110	13	
SLC39A8					
rs10014145	AA[a]	155	110 - 210	61	1.0
	AG/GG	158	120 - 180	11	
	AA[b]	155	97 - 280	61	0.2
	AG/GG	145	110 - 230	11	
rs233804	CC[a]	**157**	**110 - 210**	**65**	**0.049**
	CA/AA	**140**	**120 - 160**	**7**	
	CC[b]	155	97 - 280	65	0.3
	CA/AA	145	110 - 180	7	

[a] Probe ILMN_1695316.
[b] Probe ILMN_2233539.

Table 6 (supplementary material). Spearman´s rho correlation between expression of *SLC39A8* and metal biomarkers in a subset of the Andean population, both for total expression (A) and per genotype of rs10014145 and rs233804 (B).

A. Total expression		B-Cd	U-Cd	P-Zn	ferritin
SLC39A8[a]	r_S	-0.02	-0.07	-0.09	-0.06
	P	0.9	0.6	0.5	0.6
	N	72	72	64	70
SLC39A8[b]	r_S	0.08	0.00	0.02	-0.22
	P	0.5	0.9	0.9	0.07
	N	72	72	64	70
B Expression by SNP/Genotype					
rs10014145					
AA[a]	r_S	0.01	-0.09	-0.13	-0.01
	P	0.9	0.5	0.3	0.9
	N	61	61	54	60
AG/GG[a]	r_S	-0.19	0.04	0.05	-0.26
	P	0.6	0.9	0.9	0.5
	N	11	11	10	10
AA[b]	r_S	0.10	-0.01	-0.01	-0.21
	P	0.5	0.9	0.9	0.1
	N	61	61	54	60
AG/GG[b]	r_S	0.02	0.31	0.08	-0.15
	P	0.9	0.4	0.8	0.7
	N	11	11	10	10
rs233804					
CC[a]	r_S	0.05	-0.10	-0.12	-0.08
	P	0.7	0.4	0.4	0.5
	N	65	65	57	63
CA/AA[a]	r_S	0.14	0.00	0.40	0.11
	P	0.8	1.0	0.4	0.8
	N	7	7	7	7
CC[b]	r_S	0.18	-0.01	0.01	-0.22
	P	0.1	0.9	0.9	0.08
	N	65	65	57	63
CA/AA[b]	r_S	-0.43	-0.18	0.05	-0.43
	P	0.3	0.7	0.9	0.3
	N	7	7	7	7

[a] Probe ILMN_1695316.
[b] Probe ILMN_2233539.