Polymorphisms in Genes Encoding Potential Mercury Transporters and Urine Mercury Concentrations in Populations Exposed to Mercury Vapor from Gold Mining.

Engström, Karin; Ameer, Shegufta; Bernaudat, Ludovic; Drasch, Gustav; Baeuml, Jennifer; Skerfving, Staffan; Bose-O'Reilly, Stephan; Broberg Palmgren, Karin

Published in:
Environmental Health Perspectives

DOI:
10.1289/ehp.1204951

2013

ehp ENVIRONMENTAL HEALTH PERSPECTIVES

ehponline.org

Polymorphisms in Genes Encoding Potential Mercury Transporters and Urine Mercury Concentrations in Populations Exposed to Mercury Vapor from Gold Mining

Karin Engström, Shegufta Ameer, Ludovic Bernaudat, Gustav Drasch, Jennifer Baeuml, Staffan Skerfving, Stephan Bose-O'Reilly, Karin Broberg

http://dx.doi.org/10.1289/ehp.1204951

Online 9 October 2012

NIEHS
National Institute of Environmental Health Sciences

National Institutes of Health
U.S. Department of Health and Human Services

Polymorphisms in Genes Encoding Potential Mercury Transporters and Urine Mercury Concentrations in Populations Exposed to Mercury Vapor from Gold Mining

Karin Engström[1], Shegufta Ameer[1], Ludovic Bernaudat[2], Gustav Drasch[3], Jennifer Baeuml[4], Staffan Skerfving[1], Stephan Bose-O'Reilly[4,5], Karin Broberg[1]

[1] Department of Laboratory Medicine, Division of Occupational and Environmental Medicine, Lund University, Lund, Sweden

[2] United Nations Industrial Development Organization, Vienna, Austria

[3] Institute of Forensic Medicine, Ludwig-Maximilians-University, Munich, Germany

[4] Institute of Public Health, Medical Decision Making and Health Technology Assessment, UMIT – University for Health Sciences, Medical Informatics and Technology, Hall i.T., Austria

[5] Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University Hospital Munich, Munich, Germany

Corresponding author: Karin Engström, karin.engstrom@med.lu.se, Occupational and Environmental Medicine, Lund University Hospital, SE-22185 Lund, Sweden. Telephone: +46 (0) 46 173135. Fax: +46 (0) 46 143702.

Short running title: Genetics and toxicokinetics of inorganic Hg.

Key words: gold mining, inorganic mercury, LAT1, MDR1, MRP1, OAT1, OAT3, SLC3A2, SLC22A6, SLC22A8

Acknowledgements: The study was supported by the Swedish Council for Working Life and Social Research, the Medical Faculty of Lund University, the County Councils of Southern Sweden, and the European Union (EU; FP6; PHIME; FOOD-CT-2006-016253). The paper reflects only the authors' views; the EU is not liable for any use that may be made of the information. The samples are derived from the Global Mercury Project (GMP) implemented by the United Nations Industrial Organization (UNIDO). Thanks to Karin Paulsson for help with the genetic analyses. The authors have no competing financial interests.

Abbreviations:

BMI	body mass index
GM	geometric mean
Hg	mercury
LD	linkage disequilibrium
SNP	single nucleotide polymorphism
U-Hg	urinary mercury concentrations

Abstract

Background: Elemental mercury (Hg^0) is widely used in small-scale gold mining. Individuals working or living in mining areas have high urinary concentrations of Hg (U-Hg). Differences in genes encoding potential Hg-transporters may affect uptake and elimination of Hg.

Objective: To identify single nucleotide polymorphisms (SNPs) in Hg-transporter genes that modify U-Hg.

Methods: 1,017 men and women from Indonesia, the Philippines, Tanzania, and Zimbabwe were classified either as controls (no Hg exposure from gold mining) or as having low (living in a gold-mining area) or high exposure (working as gold miners). U-Hg was analyzed by cold-vapor atomic absorption spectrometry. Eighteen SNPs in eight Hg-transporter genes were analyzed.

Results: U-Hg concentrations were higher among *ABCC2*/*MRP2* rs1885301 A-allele carriers than among GG homozygotes in all populations, though differences were not statistically significant in most cases. *MRP2* SNPs showed particularly strong associations with U-Hg in the subgroup with highest exposure (miners in Zimbabwe) where rs1885301 A-allele carriers had higher U-Hg than GG homozygotes (geometric mean (GM): 36.4 μg/g creatinine vs. 21.9; p=0.027), rs2273697 GG homozygotes had higher U-Hg than A-allele carriers (GM: 37.4 vs. 16.7; p=0.001), and rs717620 A-allele carriers had higher U-Hg than GG homozygotes (GM: 83 vs. 28; p=0.084). The *SLC7A5*/*LAT1* rs33916661 GG genotype was associated with higher U-Hg in all populations (statistically significant for all Tanzanians combined). SNPs in *SLC22A6*/*OAT1* (rs4149170) and *SLC22A8*/*OAT3* (rs4149182) were associated with U-Hg mainly in the Tanzanian study groups.

Conclusions: SNPs in putative Hg-transporter genes may influence U-Hg concentrations.

Introduction

Mercury (Hg) is a ubiquitous and very reactive toxic metal. One of the most important sources of inorganic Hg exposure is gold mining, as elemental Hg (Hg^0) is widely used for gold extraction in small-scale gold mining (United Nations Environment Programme 2002). Hg^0 is well absorbed in the lungs and has a high neurotoxic potential, due to its ability to pass the blood-brain barrier (Nordberg et al. 2007). Hg^0 is oxidized into mercuric Hg (Hg^{2+}) in red blood cells and other tissues (Nordberg et al. 2007). Gold miners are at risk of mercury poisoning (Baeuml et al. 2011b; Bose-O'Reilly et al. 2010a, 2010b; Drasch et al. 2001, Harari et al. 2012, Lettmeier et al. 2010), and Hg^0 pollution from the gold-extraction process may also affect the health of individuals living close to mining areas (Bose-O'Reilly et al. 2010a, 2010b).

It is estimated that about 10–15 million individuals work as gold miners, and approximately 30–50 million people live in small-scale gold-mining areas (Spiegel et al. 2005). Earlier studies in the Philippines (Drasch et al. 2001), Indonesia (Bose-O'Reilly et al. 2010a), Tanzania (Bose-O'Reilly et al. 2010b), Zimbabwe (Lettmeier et al. 2010), and Ecuador (Harari et al. 2012) have shown that many individuals who work or live in gold mining areas are highly exposed to inorganic Hg from gold mining, as can be seen from high concentrations of Hg in urine (U-Hg). However, large differences in U-Hg levels among individuals with similar exposures have been reported.

Hg^{2+} formed from Hg^0 is bound to glutathione and is excreted mainly in the urine. In contrast, there is little urinary excretion of methyl-Hg, a Hg species for which exposure is mainly by consumption of fish. Hence, U-Hg is therefore mainly a biomarker of exposure to inorganic Hg (Hg^0 and Hg^{2+}). Levels of U-Hg associated with detectable health effects in exposed workers fall between 10 and 30 μg/L (1 μg/L ≈ 1 μg/g creatinine) (Nordberg et al. 2007). U-Hg levels are lower in those who are not occupationally exposed to mercury. For example, people in the Baltic region without amalgam fillings and with normal fish consumption had U-Hg levels < 1 μg/L, while those who had dental amalgams had a few μg/L of U-Hg, however concentrations up to 50 ug/L have been reported for individuals with many fillings (Skerfving et al. 1999).

As with other metals (Nordberg et al. 2007), genetics probably influences Hg uptake, distribution, and excretion. The few genetic variants that have been associated with Hg toxicokinetics identified to date have mainly been in glutathione-related genes (Custodio et al. 2005; Harari et al. 2012; Schlawicke Engstrom et al. 2008).

The different forms of Hg is thought to be transferred across cell membranes—either into or out of cells—by transporter proteins. The capacity of specific proteins for Hg transport has not been extensively studied and no specific Hg transporters have been identified. However, Hg forms complexes with small molecules such as amino acids that can mimic essential molecules recognized by transporter proteins. Some multispecific transporter families, such as organic anion transporters (OATs), system L-amino acid transporters (LAT), and ATP-binding cassette transporters (ABC transporters) are thought to alter Hg toxicokinetics (Bridges and Zalups 2005).

OATs are multispecific transport proteins located on the basolateral membrane. They mediate the uptake of a variety of substrates from renal blood. In rats, OAT1 and OAT3 have been shown to be responsible for over 60% of the renal tubular uptake of Hg^{2+} from renal blood into proximal tubule epithelial cells (Bridges and Zalups 2005).

LAT members transport amino acids into cells in exchange for other amino acids. LAT1 and LAT2 can transport cysteine-bound methyl-Hg, a complex that mimics certain amino acids (Bridges and Zalups 2005). LATs require interaction with SLC3A2 to function as transporters (del Amo et al. 2008), but nothing is known about their involvement in inorganic Hg transport.

ABC transporters participate in the transport of numerous toxicants across membranes, including anticancer agents and metals, and are major candidate genes for Hg transport. ABCC1, ABCC2, and ABCB1 (also known as multidrug resistance-associated proteins: MRP1, MRP2 and MDR1), are the most well-characterized ABC transporters. Upregulation of ABC transporters by Hg^{2+} has been demonstrated in canine kidney cells (Aleo et al. 2005). ABCC2/MRP2 has been shown to mediate excretion of Hg^{2+} from proximal tubular cells into urine in rats (Bridges et al. 2011).

The aim of the present study was to estimate associations between single nucleotide polymorphisms (SNPs) in potential Hg-transporter genes and U-Hg as an indication of possible effects of the SNPs on the toxicokinetics of inorganic Hg.

Methods

Study subjects

Participants included in the present analysis (N=1,017) were gold miners, individuals living in gold-mining areas, or control individuals living in areas with no gold mining, including men and women > 12 years of age from the Philippines (N=245) (Drasch et al. 2001), Indonesia (N=330) (Bose-O'Reilly et al. 2010a), Tanzania (N=226) (Bose-O'Reilly et al. 2010b), or Zimbabwe (N=216) (Lettmeier et al. 2010) who were examined during 2002–2007 as a part of the United Nations Industrial Development Organization's (UNIDO's) Global Mercury Project. Former gold miners and one of two Indonesian study groups from the original study were excluded due to potential misclassification of Hg exposure (Bose-O'Reilly et al. 2010a) (those excluded were not part of the 1,017 participants). Only one participant had amalgam fillings, as determined by visual inspection. Spot urine samples were collected and acidified. Blood for DNA extraction was collected in EDTA tubes.

All participants were volunteers who provided written informed consent. The health examinations were performed in close cooperation with UNIDO and the United Nations Development Programme, as well as the regional health authorities and national ministries of health. All conditions were agreed upon between the governments and UNIDO, including extensive legal, formal, and ethical considerations. The conditions were approved by the governments and complied with all relevant national, state, and local regulations.

Categories and biomarkers of Hg exposure

Based on a questionnaire, the participants were divided into three exposure subgroups: (1) controls (with no particular Hg exposure from gold mining, N=139), (2) low-exposure individuals (living in Hg-contaminated mining areas, N=297), and (3) high-exposure individuals (gold miners working with Hg^0, N=581). Participants in control subgroups were living in areas without any gold mining. Participants in the low-exposure subgroups were living in gold mining areas and were exposed to the Hg-contaminated environment. Participants in the high-exposure subgroups were living in the same areas as the low-exposure participants and were working as miners with immediate contact with Hg, such as panning the crushed ore together with liquid Hg, or smelting the amalgams. Six of the 1,017 study participants did not have data on urinary mercury.

All U-Hg analyses were performed in the Institute of Forensic Medicine (LMU). Urine was analyzed for total Hg by cold-vapor atomic absorption spectrometry using a Perkin-Elmer 1100 B spectrometer after capturing the mercury on a gold amalgam net, with a MHS 20 attachment. Sodium-boro hydride was applied for reduction of Hg. The detection limit was 0.20 µg/L. For results below the limit of detection, the value was set to half the detection limit. U-Hg was adjusted for creatinine, analyzed by the Jaffe method (Mazzachi et al. 2000). Strict internal and external quality control was ensured. Quality controls with reference urine samples (ClinCal® urine (41 µg Hg/L) and ClinCeck® urine level I (3.5 µg Hg/L) and II (40 µg Hg/L) for trace elements (RECIPE Chemicals + Instruments GmbH, Munich, Germany) were continuously performed to ensure the accuracy of the results. The laboratory has demonstrated good performance in external quality control tests for mercury in human specimens. The laboratory has participated in round robin tests by the German DGKL ("Deutsche Vereinte Gesellschaft für klinische Chemie und Laboratoriumsmedizin e.V.) for Hg in biological samples since 1998.

Genotyping

DNA was extracted from whole blood using the Qiagen DNA Blood Mini kit (Qiagen, Hilden, Germany). We selected SNPs based on three different criteria: (1) functional impact according to the literature; (2) potential functional impact according to position and type of SNP (specifically, non-synonymous SNPs that may affect the protein structure/enzyme activity or 5' SNPs at putative promoter sites that can affect gene expression) (NCBI 2006); and (3) tagSNPs that capture as much of the genetic variation within a gene segment as possible due to linkage disequilibrium (LD) with other SNPs. TagSNPs were selected according to HapMap data (Thorisson et al. 2005) for YRI (Yoruba in Ibadan, Nigeria) as a proxy for the African populations, and CHB (Han Chinese in Beijing, China) and JPT (Japanese in Tokyo, Japan) for Asians. In total, 18 SNPs were analyzed in genes coding for eight different transporters (Table 1). Genotyping was done using the iPLEX® Gold assay on the MassARRAY platform (Sequenom^{TM}) for 15 SNPs. Additionally, three SNPs in *MRP2* (rs717620, rs2273697, and rs3740066) were analyzed by Taqman allelic discrimination assay on an ABI 7900 instrument (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. Genotyping failed for 2 SNPs (*MRP1* rs41395947 and *SLC3A2* rs2282477); for the other SNPs, Sequenom^{TM} genotyping failed for ≤ 2% and Taqman genotyping failed for ≤ 4% of study participants. Five percent of the samples were re-analyzed for quality control purposes. There was perfect agreement between original and repeat genotyping runs for all SNPs.

Bioinformatics

Potential functional effects of SNPs with at least one statistically significant association ($p < 0.05$) with U-Hg in at least one study group were evaluated in silico. Promoter sites and

potential transcription factor binding sites were analyzed by Genomatix software suite (Genomatix Software GmbH, Munich, Germany). This was evaluated for SNPs situated in the 5´ UTR or introns.

Statistical analysis

In each country, deviations from Hardy-Weinberg equilibrium were tested using chi-square analysis and LD was evaluated using Haploview (Barrett et al. 2005).

Natural log (ln) transformed U-Hg was the dependent variable. The general linear model procedure was used, in which genotypes with three levels can be modeled as nominal variables without assuming additive effects.

First, we estimated genetic associations with U-Hg for all individuals in a country with adjustment for exposure subgroup (controls, low or high exposure) as categorical (nominal) variables, and after stratification by low or high exposure subgroup. In addition, we estimated associations adjusted for exposure subgroup according to continent (Africa, including Tanzania and Zimbabwe; or Asia, including Indonesia and the Philippines). We refer to these models as Model 1. Numbers of participants in the control groups were too small for stratum-specific analyses. Genotypes were modeled as categorical (nominal variables) coded 0, 1 or 2 according to the number of variant alleles, or were dichotomized with the common homozygous genotype. The genotype denoted last in the text in the Results section (first column in the tables) was used as reference (the common homozygote or the combination of the heterozygote and the common homozygote). However, in order to facilitate the interpretation of the results, other genotypes were sometimes used as reference, based on effect estimates.

Next, we estimated associations between genotypes (categorical, as above) and U-Hg by country or continent with adjustment for age (continuous) and gender, in addition to adjusting for exposure subgroup (categorical) or stratification according to low or high exposure (Model 2). In the analyses stratified for exposure group, adjustments were also made for Hg storage (categorical, categorized as never, at work, or at home). We did not include Hg storage in the analyses by country or continent because of strong co-linearity with exposure subgroup (Pearson product-moment correlation coefficient, r=0.8). Alcohol intake (never, > once a month, > once a week, or > once a day based on their response to the question "do you drink alcohol?", or categorized as "yes" or "no" for the Philippines) and BMI (continuous) were not included in models because they were correlated with gender (r around 0.5).

Statistical analyses were performed using SPSS (version 18; SPSS, Chicago, IL, USA). Statistical significance denotes $p<0.05$ (two-tailed).

Results

Background data

The study groups with the highest and lowest geometric mean (GM) creatinine-adjusted U-Hg concentrations were from Zimbabwe and Tanzania, respectively (Table 2). Correlations (r) between U-Hg and potentially influential variables in the different populations are shown in Supplemental Material, Table S1. U-Hg was significantly correlated (all $p<0.001$) with exposure subgroup (r=0.46–0.85), Hg storage (r=0.33–0.72), and male gender (r=0.22–0.37) in all 4 countries, but was only weakly correlated with age (r= -0.12–0.093, $p>0.05$). There

was a positive correlation between U-Hg and alcohol consumption (r=0.14–0.33, p-values of <0.050; data not available for the Philippines) and positive correlations between alcohol consumption and other factors such as gender and exposure group (r=0.3–0.5).

Genotyping was successful for 16/18 SNPs (Table 1). The minor alleles were the same in each country, for all SNPs (calculated for the total study population from each country, including the controls) except for *MDR1* rs2032582 in the Philippines. There were few differences in allelic frequencies among the different exposure subgroups within each country (Fisher's exact test, not in table). In Indonesia, the allele frequency for the control group (N=19) deviated from the low exposed for one SNP (rs717620). All SNPs were in Hardy-Weinberg equilibrium in all countries, except for *MRP2* rs717620 in Indonesia (Chi-square 7.1, $p<0.05$) and Philippines (Chi-square 23, $p<0.05$).

SNPs that were statistically significantly associated with U-Hg (*MRP2*, *OAT1*, *OAT3* and *LAT1*) in at least one country/subgroup are presented in the results section. For the other SNPs evaluated, no statistically significant associations with U-Hg were found.

Unstandardised β-coefficients derived from the comparison with the reference genotype, i.e., the estimated difference in ln-transformed U-Hg for two genotypes, and corresponding p-values and standard error (SE) are presented (Table 3 for *MRP2* and Table 4 for *LAT1*, *OAT1* and *OAT3*). P-value for the genotype term in the model are presented in the tables as well as when specifically stated in the text. All above are derived from Model 2 (adjusted model) if not stated otherwise.

MRP2 and U-Hg

The *MRP2* rs1885301 A-allele carriers had higher observed creatinine-adjusted geometric mean U-Hg concentrations than those with the GG genotype in most populations (overall and according to high or low exposure within each country) (Supplemental material, Table S2). When we pooled data from the Asian or African countries respectively, a significant association with U-Hg was seen for Asia (p=0.002, β=0.33, SE=0.11, AA+AG vs. GG), but not for Africa (p=0.30, β=0.17, SE=0.17, AA+AG vs. GG).

Bioinformatic analyses indicated that rs1885301 is situated 5' of the promoter and that the G>A substitution results in loss of one transcription-factor binding site (Fetal Alz-50 clone 1 (FAC1) and gain of another site (FAST-1 SMAD interacting protein).

MRP2 rs717620 was rare in the African countries, where frequencies of the A-allele were only 1%, as compared to 15–20% in Asia. Individuals with one or two copies of the A-allele had higher U-Hg concentrations in all exposure subgroups in which at least four individuals carried this allele (Supplemental Material, Table S2). This was statistically significant in the Philippines (p=0.041, β=0.33, SE=0.16, AA vs. AG+GG). In the pooled analyses, associations were in the same directions: individuals with one or two copies of the A-allele had higher U-Hg concentrations (Asia: p=0.010, β=0.31, SE=0.12, AA vs. AG+GG; Africa: p=0.26, β=0.59, SE=0.53, AA vs. AG+GG). As noted above, this SNP was in Hardy-Weinberg disequilibrium in the Asian populations. In silico analysis indicated that rs717620 is situated in a promoter site, and that the G>A substitution leads to loss of two transcription-factor binding sites (LEF-1 and SOX4).

The non-synonymous *MRP2* rs2273697 was significantly associated with U-Hg among high exposed miners in Zimbabwe only, such that carriers of the A-allele (Ile) had lower U-Hg concentrations than individuals with no copies of the A-allele (p=0.001, β=-0.78, SE=0.22, AA+AG vs. GG). The A-allele was also associated with lower U-Hg among high-exposed miners in the Philippines (who had the second-highest U-Hg among all population subgroups), although this association was not statistically significant (p=0.55, β=-0.19, SE=0.33, AA+AG vs. GG), as well as among all African participants combined (p=0.025, β=-0.40, SE=0.18, AA+AG vs. GG). However, the association was in the opposite direction in Indonesia (all individuals), although not statistically significant (p=0.20, β=0.41, SE=0.32, AA+AG vs. GG).

Overall, associations with the three *MRP2* SNPs discussed above were particularly strong in the highest exposed subgroup, gold miners in Zimbabwe. In this subgroup, rs1885301 A-allele carriers had higher U-Hg than those with the GG genotype (observed GM: 36.4 vs. 21.9 µg/g creatinine, Model 1 unadjusted p=0.075, β=0.51, SE=0.23, AA+AG vs. GG), rs2273697 GG genotype had higher U-Hg concentrations than A-allele carriers (observed GM: 16.7 vs.37.4 µg/g creatinine, Model 1 unadjusted p=0.001, β=0.81, SE=0.24, AA+AG vs. GG), and rs717620 A-allele carriers had higher U-Hg concentrations than those with the GG genotype (observed GM 83.0 vs.28 µg/g creatinine, Model 1 unadjusted p =0.084, β=1.1, SE=0.63, AA+AG vs. GG).

Rs2273697 was not in linkage disequilibrium (LD) with rs1885301 or rs717620 in any population. Rs717620 was in LD with rs1885301 (the common G-alleles of both SNPs were linked to each other) in Indonesia (R^2=0.69) and the Philippines (R^2=0.40), but not in the African study groups (R^2=0.02 in both countries).

LAT1 and U-Hg

For *LAT1* rs33916661, individuals with the rare GG genotype showed higher observed GM U-Hg concentrations than individuals carrying GA or AA genotypes when all participants were combined by country or continent as well as in the high exposed subgroups (Supplemental Material, Table S3). However, the associations were statistically significant only in Tanzania (Table 4, p=0.015 for the genotype term in the adjusted models, p=0.064, β=-0.51, SE=0.27 for the comparison of AA vs. GG and p=0.004, β=-0.79, SE=0.28 for AG vs. GG) and when the African countries were pooled (p=0.046 for the genotype term, p=0.024, β=-0.59, SE=0.26 for AA vs. GG, p=0.017, β=-0.63, SE=0.26 for AG vs.GG). In Tanzania (all individuals) and in the African group, the group with GG genotype had about twice the GM U-Hg concentration as those with AG or AA genotypes (Supplemental Material, Table S3). This SNP is situated in the 5' untranslated region, and in silico analysis indicated that it results in the loss of two transcription-factor binding sites: Paired box 5 B-cell-specific activator protein new (PAX5), and Avian C-type LTR CCAAT box binding factors, Zinc finger protein 217 (ZNF217).

OATs and U-Hg

Two of three SNPs evaluated in OATs (*OAT1* rs4149170 and *OAT3* rs4149182) were associated with lower U-Hg in the African populations (Table 4). These genotypes were rare in the Asian populations (2–4%). In most cases, individuals with the *OAT1* rs4149170 AA or the *OAT3* rs4149182 CC genotype had lower GM U-Hg concentrations than those with AG or GG and CG or GG genotypes, respectively (Supplemental Material, Table S3).

Statistically significant associations were seen in all groups in Tanzania: individuals with AA or CC genotypes had lower U-Hg concentrations than the GG genotypes (reference). For rs4149170, p-values for the genotype term were 0.025 (low exposed), 0.024 (high exposed) and 0.023 (all individuals). When comparing AA with GG: p=0.022, β=-0.79, SE=0.33 for low exposed, p=0.039, β=-0.70, SE=0.34 for high exposed, and p=0.024, β=-0.54, SE=0.24 for all individuals. For rs4149182, p-values for the genotype term were 0.004 (low exposed), 0.059 (high exposed), and 0.017 (all individuals). When comparing CC with GG: p=0.002, β=-1.05, SE=0.32 for low exposed, p=0.046, β=-0.69, SE=0.34 for high exposed, and p=0.010, β=-0.62, SE=0.24 for all individuals. In these groups, the U-Hg concentrations were about 50% lower for individuals with AA or CC compared to GG (Supplemental Material, Table S3). Individuals with AA or CC genotypes had lower U-Hg in Zimbabwe, although these associations did not reach statistical significance. No statistically significant associations were seen for these SNPs when the African or Asian study groups were pooled. *OAT1* rs4149170 and *OAT3* rs4149182 were in LD in the African populations (the common G-alleles of both SNPs were linked to each other: Tanzania, R^2=0.82; Zimbabwe, R^2=0.55) but this was not found in the Asian populations (Indonesia, R^2=0.06; Philippines, R^2=0.01). In silico analyses indicate that rs4149182 in *OAT3* is situated in a binding site for metal-regulatory transcription factor 1 (MTF1), and that the C>G transversion results in loss of the MTF1-binding site.

As there were correlations between alcohol consumption and gender, and BMI and gender, we did not include them in the models above in order to avoid over-adjustment. However, we conducted a sensitivity analysis and compared model estimates adjusted for either alcohol consumption or BMI instead of gender. Results were generally consistent with those adjusted for gender (data not shown). Since gender was correlated with U-Hg concentrations we also

compared associations between men and women by including an interaction term (gene x gender) in the models or by stratifying by gender in addition to the stratifications for country/continent and exposure group. However, no clear patterns could be seen (data not shown), probably due to a too low power.

Discussion

In this study, we report evidence suggesting that variations in the transporter gene *MRP2* influence U-Hg in individuals exposed to elemental Hg. Associations between *MRP2* rs1885301 and U-Hg were observed in study populations from two continents, and associations with two *MRP2* SNPs (rs1885301 and rs2273697) appeared to be particularly strong in gold miners in Zimbabwe, the study subgroup with highest exposure. Polymorphisms in *LAT1, OAT1*, and *OAT3* were associated with U-Hg, but statistically significant associations were mainly observed in Tanzania. In silico analyses indicated that several of the SNPs associated with U-Hg alter putative transcription-factor binding sites, which indicate that they may influence gene expression.

This study had several strengths. The exposed populations were fairly large, ranging between 171 and 309 individuals in the different countries, although there were relatively few participants in the control populations that were not exposed to elemental mercury used in gold mining. U-Hg determinations and genotyping were done by the same methods in the same laboratories, and with good quality control. U-Hg concentrations indicated a wide range of exposures (with GM in the different exposure groups ranging from 0.12 to 29 μg/g creatinine in Zimbabwe participants, for example.)

For some polymorphisms, however, the frequencies were low, which limited our ability to identify associations or consistent patterns across populations. We cannot exclude the possibility that differences in genetic background between exposure subgroups within each country might exist for other genes that influence Hg metabolism, since controls were not always living in exactly the same area, and gold miners were work migrants in many cases.

The allelic frequencies varied among the populations, as did the LD patterns (data not shown). Thus, we performed separate analyses for each country. We had a problem of multiple inference. However, the main candidate gene, well-founded in the literature, was *MRP2*, while associations with U-Hg for the other genes evaluated were plausible but less well supported. To increase our power to detect associations we merged the African and Asian populations in separate analyses. These results must be interpreted with caution due to differences in mining practices that may have influenced exposure levels, including differences in frequency and intensity of gold-amalgam burning, which is the main route of Hg^0 exposure (Harari et al. 2012). The Asian countries were more similar in exposure and U-Hg concentrations than the African ones, and thus provided the strongest results in the merged analyses. There were no large variations in allele frequencies between the countries within the pooled Asian and African groups.

Hg excretion among gold miners varies over time, in association with the time since the last burning of gold amalgam (Harari et al. 2012), which could obscure genetic effects, if present. This may explain why associations with SNPs in *OAT1, OAT3* and *LAT1* mainly were seen in Tanzania, where the exposure was lowest, but was probably the most stable over time.

Alcohol intake may modify the levels of U-Hg. Alcohol consumption has been shown to increase the rate of exhalation of mercury and to reduce U-Hg levels (Clarkson and Magos 2006). However, we decided not to include it in the statistical models in order to avoid over-adjustment; alcohol intake was positively associated with both exposure group and gender. The positive correlation between alcohol consumption and U-Hg in our study populations was inconsistent with expectations, which makes us believe that the association was confounded by a positive association between alcohol consumption and exposure group/gender rather than being a true effect.

Gender differences in metal toxicokinetics and toxicodynamics have been described for some metals, including methylmercury (Vahter et al. 2007). Gender was correlated with U-Hg in our study populations, but was also associated with other factors related to exposure (alcohol consumption, BMI, work tasks - men were more likely than women to report that they worked with smelting amalgam). We did not find any gene-gender interactions for U-Hg, but we had limited power for these analyses, especially in the high-exposed groups where women constituted 20-30% of the study participants.

Transporter proteins are involved in active uptake of compounds, and as the Hg uptake in the lung is through passive absorption, we believe it is unlikely that lung absorption of Hg^0 is affected by transporter genes (Nordberg et al. 2007). Our hypothesis is that, given the same exposure, individuals with higher U-Hg would have enhanced excretion.

MRP2 is an important regulator of metal toxicity in different species, such as in rats and *Arabidopsis thaliana* (Liu et al. 2001; Song et al. 2010). Hg induces upregulation of *MRP2* and expression of MRP2 protein in vitro (Aleo et al. 2005). MRP2 transports Hg out of cells and MRP2 levels are high in the kidney (Wu et al. 2009). Increased MRP2 expression in the kidney may increase urinary Hg excretion. Two of the *MRP2* SNPs that we evaluated (rs1885301 and rs717620) are situated 1,500 base pairs apart, both at the 5' end of the gene, which is associated with regulation of gene expression. However, there are no data for the influence of the gene expression for rs1885301. For rs717620, the data are inconclusive; no effect of the variant A-allele was found on gene or protein expression levels in liver cells or enterocytes in three studies analyzing expression (Deo et al. 2012, Moriya et al. 2002, Zhang et al 2010), whereas lower mRNA levels in kidney tissue were reported in one study (Haenisch et al. 2007). Since the in silico analysis showed a loss of two activating transcription factors for the rs717620 A-allele, this finding is in line with the lower mRNA levels found in Haenisch et al. We did not identify any reports concerning effects of *MRP2* SNPs on metal transport.

Previously, LAT1 has only been shown to transport methyl-Hg in *Xenopus laevis* oocytes (Simmons-Willis et al. 2002). *LAT1* rs33916661 is situated at the 5' end of the gene. Little is known about the functional impact of this polymorphism. Our in silico analysis suggests that the GG genotype, which was associated with higher U-Hg concentrations in most population subgroups, results in the loss of two transcription-factor binding sites and a gain of one new site.

OAT1 and OAT3 mediate uptake of Hg^{2+} into cells, particularly in the kidney (Bridges and Zalups 2005). Rs4149182 was found to be situated in an MTF1-binding site, a so-called metal-responsive element. MTF1 induces a positive regulation of transcription in response to metal ions. The C>G transversion resulted in loss of an MTF1-binding site.

Further functional studies are, however, needed to prove causation between the SNPs identified and Hg metabolism. False-positive results may have arisen due to multiple testing of several genetic markers in different populations. Furthermore, the SNPs identified may not be functional, but may be linked to other unknown and truly functional SNPs. There is also a problem with misclassification of exposure subgroups, as the miners were placed in the same category according to their working circumstances and not based on true exposure levels.

Conclusions

Several SNPs in genes coding for transporters were associated with U-Hg concentrations in at least some study populations. Results support the hypothesis that transporters may be involved in the toxicokinetics of Hg. In particular, some *MRP2* variants were associated with U-Hg in study groups from different countries.

References

Aleo MF, Morandini F, Bettoni F, Giuliani R, Rovetta F, Steimberg N, et al. 2005. Endogenous thiols and MRP transporters contribute to Hg2+ efflux in HgCl2-treated tubular MDCK cells. Toxicology 206:137-151.

Baeuml J, Bose-O'Reilly S, Lettmeier B, Maydl A, Messerer K, Roider G, et al. 2011a. Applicability of two mobile analysers for mercury in urine in small-scale gold mining areas. Int J Hyg Environ Health 215:64-67.

Baeuml J, Bose-O'Reilly S, Matteucci Gothe R, Lettmeier B, Roider G, Drasch G, et al. 2011b. Human Biomonitoring Data from Mercury Exposed Miners in Six Artisanal Small-Scale Gold Mining Areas in Asia and Africa. Minerals 1:122-143.

Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263-265.

Bose-O'Reilly S, Drasch G, Beinhoff C, Rodrigues-Filho S, Roider G, Lettmeier B, et al. 2010a. Health assessment of artisanal gold miners in Indonesia. Sci Total Environ 408:713-725.

Bose-O'Reilly S, Drasch G, Beinhoff C, Tesha A, Drasch K, Roider G, et al. 2010b. Health assessment of artisanal gold miners in Tanzania. Sci Total Environ 408:796-805.

Bridges CC, Joshee L, Zalups RK. 2011. MRP2 and the handling of mercuric ions in rats exposed acutely to inorganic and organic species of mercury. Toxicol Appl Pharmacol 251:50-58.

Bridges CC, Zalups RK. 2005. Molecular and ionic mimicry and the transport of toxic metals. Toxicol Appl Pharmacol 204:274-308.

Clarkson TW, Magos L. 2006. The toxicology of mercury and its chemical compounds. Crit Rev Toxicol 36:609-662.

Custodio HM, Harari R, Gerhardsson L, Skerfving S, Broberg K. 2005. Genetic influences on the retention of inorganic mercury. Arch Environ Occup Health 60:17-23.

del Amo EM, Urtti A, Yliperttula M. 2008. Pharmacokinetic role of L-type amino acid transporters LAT1 and LAT2. Eur J Pharm Sci 35:161-174.

Deo AK, Prasad B, Balogh L, Lai Y, Unadkat JD. 2012. Inter-individual Variability in Hepatic Expression of the Multidrug Resistance-associated Protein 2 (MRP2/ABCC2): Quantification by LC/MS/MS. Drug Metab Dispos 40:852-5.

Drasch G, Bose-O'Reilly S, Beinhoff C, Roider G, Maydl S. 2001. The Mt. Diwata study on the Philippines 1999--assessing mercury intoxication of the population by small scale gold mining. Sci Total Environ 267:151-168.

Haenisch S, Zimmermann U, Dazert E, Wruck CJ, Dazert P, Siegmund W, et al. 2007. Influence of polymorphisms of ABCB1 and ABCC2 on mRNA and protein expression in normal and cancerous kidney cortex. Pharmacogenomics J 7:56-65.

Harari R, Harari F, Gerhardsson L, Lundh T, Skerfving S, Stromberg U, et al. 2012. Exposure and toxic effects of elemental mercury in gold-mining activities in Ecuador. Toxicol Lett. 213:75-82.

Lettmeier B, Boese-O'Reilly S, Drasch G. 2010. Proposal for a revised reference concentration (RfC) for mercury vapour in adults. Sci Total Environ 408:3530-3535.

Liu J, Chen H, Miller DS, Saavedra JE, Keefer LK, Johnson DR, et al. 2001. Overexpression of glutathione S-transferase II and multidrug resistance transport proteins is associated with acquired tolerance to inorganic arsenic. Mol Pharmacol 60:302-309.

Mazzachi BC, Peake MJ, Ehrhardt V. 2000. Reference range and method comparison studies for enzymatic and Jaffe creatinine assays in plasma and serum and early morning urine. Clin Lab 46:53-55.

Moriya Y, Nakamura T, Horinouchi M, Sakaeda T, Tamura T, Aoyama N, et al. 2002. Effects of polymorphisms of MDR1, MRP1, and MRP2 genes on their mRNA expression levels in duodenal enterocytes of healthy Japanese subjects. Biol Pharm Bull 25:1356-1359.

NCBI. 2006. SNP Database. Available: http://www.ncbi.nlm.nih.gov/SNP [Accessed 09 Jan 2012].

Nordberg G, Fowler B, Nordberg M, Friberg L. 2007. Handbook on the toxicology of metals, third edition. Elsevier.

Schlawicke Engstrom K, Stromberg U, Lundh T, Johansson I, Vessby B, Hallmans G, et al. 2008. Genetic variation in glutathione-related genes and body burden of methylmercury. Environ Health Perspect 116:734-739.

Skerfving S, Bencko V, Vahter M, Schütz A, Gerhardsson L. 1999. Environmental health in the Baltic region-toxic metals. Scand J Work Environ Health. Suppl 3:40-64.

Simmons-Willis TA, Koh AS, Clarkson TW, Ballatori N. 2002. Transport of a neurotoxicant by molecular mimicry: the methylmercury-L-cysteine complex is a substrate for human L-type large neutral amino acid transporter (LAT) 1 and LAT2. Biochem J. 367:239-46.

Song WY, Park J, Mendoza-Cozatl DG, Suter-Grotemeyer M, Shim D, Hortensteiner S, et al. 2010. Arsenic tolerance in Arabidopsis is mediated by two ABCC-type phytochelatin transporters. Proc Natl Acad Sci U S A 107:21187-21192.

Spiegel SJ, Yassi A, Spiegel JM,Veiga MM. 2005. Reducing mercury and responding to the global gold rush. Lancet 366:2070-2072.

Thorisson GA, Smith AV, Krishnan L,Stein LD. 2005. The International HapMap Project Web site. Genome Res 15:1592-1593. Available: www.hapmap.org [Accessed 6 Mar 2012]

United Nations Environment Programme. 2002. Global mercury assessment. In. Geneva, Switzerland: UNEP (United Nations Environment Programme) Chemicals, http://www.chem.unep.ch.

Vahter M, Akesson A, Lidén C, Ceccatelli S, Berglund M. Gender differences in the disposition and toxicity of metals. 2007. Environ Res 104:85-95.

Wu C, Orozco C, Boyer J, Leglise M, Goodale J, Batalov S, et al. 2009. BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. Genome Biol 10:R130.

Zhang Y, Zhao T, Li W, Vore M. 2010. The 5'-untranslated region of multidrug resistance associated protein 2 (MRP2; ABCC2) regulates downstream open reading frame expression through translational regulation. Mol Pharmacol 77:237-246.

Table 1. Characteristics of the single nucleotide polymorphisms (SNPs)[a] under study in different countries.

Gene	ID of SNP[a]	Type of SNP[b]	Allele frequencies[b]			
			Indonesia	Philippines	Tanzania	Zimbabwe
MDR1 (ABCB1)	rs1045642	synonymous, C>A>T	63/0/37	64/0/36	88/0/12	85/0/15
MDR1	rs2032582	non-synonymous, Ala>Thr>Ser	52/5/43	43/9/48	98/0/2	100/0/0
MRP1 (ABCC1)	rs11075290	intronic, C>T	57/43	69/31	71/29	74/26
MRP1	rs41395947	non-synonymous, Cys>Ser	[c]	[c]	[c]	[c]
MRP2 (ABCC2)	rs1885301	5' UTR/ near gene, G>A	76/24	78/22	63/37	66/34
MRP2	rs717620	5' UTR/ near gene, G>A	80/20	85/15	99/1	99/1
MRP2	rs2273697	non-synonymous, Val>Ile, G>A	97/3	89/11	79/21	83/17
MRP2	rs3740066	synonymous, G>A	79/21	79/21	82/18	85/15
OAT1 (SLC22A6)	rs4149170	5' UTR, G>A	80/20	88/12	62/38	53/47
OAT1	rs11568626	non-synonymous, Pro>His>Arg>Leu	100/0/0/0	100/0/0/0	91/0/0/9	90/0/0/10
OAT3 (SLC22A8)	rs4149182	intronic, G>C	76/24	79/21	63/37	62/38
OAT3	rs45566039	non-synonymous, Arg>Ser	100/0[c]	100/0[c]	100/0[c]	100/0[c]
SLC3A2	rs2282477	3' UTR, C>T	[c]	[c]	[c]	[c]
SLC3A2	rs77030286	5' near gene, C>A	100/0	100/0	100/0	100/0
LAT1 (SLC7A5)	rs3815559	intronic, G>C	89/11	90/10	65/35	72/28
LAT1	rs33916661	5' UTR/ near gene, A>G	79/21	82/18	67/33	65/35
LAT2	rs12879346	5' UTR/ near gene, A>T	64/36	66/34	63/37	67/33
LAT2	rs12879346	5' UTR/ near gene, A>T	64/36	66/34	63/37	67/33

Abbreviation: UTR = untranslated region.

[a] Rs (reference SNP ID)-numbers are from the NCBI SNP Database (NCBI 2006).

[b] The most common allele in all four populations is given first.

[c] Data are missing due to genotyping failure.

Table 2. Characteristics of the study populations.

Characteristic	Indonesia		Philippines		Tanzania		Zimbabwe	
	N[a]		N		N		N	
Age (years)[b]	330	33 (13–58)	245	37 (23–51)	226	33 (16–51)	216	28 (14–46)
Sex	330		245		226		215	
–Female (%)		46		38		38		35
Storage of Hg (%)	325		243		224		215	
–Never		35		60		36		30
–At home		39		24		42		63
–At work		25		16		21		7
BMI (kg/m^2)[b]	310	23 (18–31)	245	24 (19–30)	223	22 (17–31)	211	21 (16–26)
Alcohol consumption	329		240		226		216	
–No (%)		86		35		74		65
Urinary Hg (µg/g creatinine)[c]								
–All	330	4.2 (0.047–95)	244	2.9 (0.40–44)	221	1.0 (0.09–15)	216	7.7 (0.91–160)
–Controls	21	0.38 (0.094–1.3)	40	2.0 (0.66–8.5)	32	0.17 (0.047–0.63)	45	0.12 (0.030–2.1)
–Low exposure	106	2.8 (0.60–15)	113	1.3 (0.28–8.9)	52	0.41 (0.082–1.8)	24	5.3 (0.43–65)
–High exposure	203	6.7 (0.91–160)	91	9.3 (1.3–61)	137	2.0 (0.20–20)	147	29 (2.3–210)

[a] Number of individuals.

[b] Mean (5th–95th percentile).

Table 3. Multivariate analyses[a] for *MRP2* SNPs on urinary mercury concentrations (µg/g creatinine)[b]: β-coefficients for each genotype with corresponding p-value and standard error (SE)[c], and model p-value[d].

Country/ continent		rs1885301				rs1885301			rs717620				rs717620			rs2273697[i]		
Exposure Subgroup[f]	p(N)[e] β(SE)	*GG*[g]	*GA*	*AA*	p mod[d]	*GG*[g]	*AA+AG*	p mod[d]	*GG*[g]	*GA*	*AA*	p mod[d]	*GG*[g]	*AA+GA*	p mod[d]	*GG*[g]	*AA+GA*[h]	p mod[d]
Indonesia																		
Low	p(N)	(54)	0.065(47)	0.15(5)	0.10	(54)	0.039(52)	0.039	(61)	0.31(42)	0.18(8)	0.32	(61)	0.18(42)	0.18	(102)	(2)	[i]
	β(SE)		0.42(0.23)	0.76(0.52)			0.46(0.22)			0.26(0.25)	0.58(0.44)			0.32(0.24)				
High	p(N)	(119)	0.14(65)	0.42(15)	0.29	(119)	0.12(80)	0.12	(135)	0.11(54)	0.10(12)	0.046	(135)	0.40(66)	0.40	(184)	0.22(17)	0.21
	β(SE)		0.34(0.23)	0.32(0.39)			0.34(0.21)			0.39(0.24)	-0.76(0.46)			0.19(0.23)			0.46(0.38)	
All	p(N)	(190)	0.075(115)	0.19(20)	0.12	(190)	0.046(135)	0.046	(213)	0.11(89)	0.52(21)	0.18	(213)	0.25(110)	0.25	(306)	0.20(19)	0.20
	β(SE)		0.28(0.16)	0.40(0.31)			0.30(0.15)			0.27(0.17)	-0.20(0.31)			0.18(0.16)			0.41(0.32)	
Philippines																		
Low	p(N)	(71)	0.11(34)	0.59(7)	0.20	(71)	0.23(41)	0.23	(76)	0.13(23)	[j]	0.13	(76)	0.13(23)	0.13	(82)	0.76(19)	0.76
	β(SE)		0.33(0.20)	-0.20(0.38)			0.23(0.19)			0.39(0.25)				0.37(0.24)			0.08(0.25)	
High	p(N)	(55)	0.70(29)	0.24(7)	0.49	(55)	0.45(36)	0.45	(51)	0.95(22)	0.51(5)	0.45	(51)	0.86(27)	0.86	(56)	0.56(22)	0.55
	β(SE)		0.11(0.29)	0.61(0.51)			0.21(0.27)			-0.02(0.33)	0.40(0.62)			0.05(0.31)			-0.19(0.33)	
All	p(N)	(148)	0.15(78)	0.33(16)	0.27	(148)	0.11(94)	0.11	(158)	0.061(49)	0.30(9)	0.12	(158)	0.041(58)	0.041	(172)	0.45(46)	0.44
	β(SE)		0.21(0.15)	0.27(0.28)			0.22(0.14)			0.33(0.17)	0.38(0.36)			0.33(0.16)			-0.13(0.18)	
Tanzania																		
Low	p(N)	(16)	0.49(27)	0.58(7)	0.75	(16)	0.44(34)	0.44	(53)	(0)	(0)	[i]	(53)	(0)	[i]	(27)	0.61(24)	0.61
	β(SE)		0.21(0.30)	0.24(0.43)			0.22(0.28)										0.14(0.28)	
High	p(N)	(54)	0.62(63)	0.93(19)	0.88	(54)	0.66(82)	0.66	(131)	(2)	(1)	[i]	(131)	(3)	[i]	(85)	0.075(46)	0.075
	β(SE)		0.13(0.26)	0.03(0.38)			0.11(0.25)										-0.45(0.25)	
All	p(N)	(85)	0.55(101)	0.92(32)	0.82	(85)	0.60(133)	0.60	(214)	0.93(5)	[j]	0.93	(214)	0.93(5)	0.93	(134)	0.40(80)	0.40
	β(SE)		0.11(0.19)	0.03(0.26)			0.09(0.17)			-0.05(0.57)				-0.05(0.57)			-0.15(0.18)	

[Table 3 continued]

Country/ continent		rs1885301				rs1885301			rs717620				rs717620			rs2273697[i]		
Exposure Subgroup[f]	p(N)[e] β(SE)	*GG*[g]	*GA*	*AA*	p mod[d]	*GG*[g]	*AA+AG*	p mod[d]	*GG*[g]	*GA*	*AA*	p mod[d]	*GG*[g]	*AA+GA*	p mod[d]	*GG*[g]	*AA+GA*[h]	p mod[d]
Zimbabwe																		
Low	p(N)	(7)	0.63(17)		0.63	(7)	0.63(17)	0.63	(24)	(0)	(0)	[i]	(24)	(0)	[i]	(17)	0.97(7)	0.97
	β(SE)		0.32(0.65)				0.32(0.65)										0.02(0.64)	
High	p(N)	(63)	0.036(65)	0.033(18)	0.030	(63)	0.013(83)	0.013	(136)	0.19(5)	[j]		(136)	0.19(5)		(95)	0.001(46)	0.001
	β(SE)		0.47(0.23)	0.71(0.33)			0.53(0.21)			0.77(0.58)		0.19		0.77(0.58)	0.19		-0.78(0.22)	
All	p(N)	(90)	0.076(103)	0.15(22)	0.14	(90)	0.049(125)	0.049	(204)	0.17(6)	[j]		(204)	0.17(6)		(147)	0.022(63)	0.022
	β(SE)		0.33(0.18)	0.43(0.30)			0.35(0.18)			0.72(0.53)	0.17	0.17		0.72(0.53)	0.17		-0.44(0.19)	
Asia	p(N)	(339)	0.004(194)	0.13(36)	0.009	(339)	0.002(230)	0.002	(373)	0.004(138)	0.92(30)	0.015	(373)	0.010(168)	0.010	(478)	0.78(65)	0.78
	β(SE)		0.33(0.11)	0.34(0.22)			0.33(0.11)			0.38(0.13)	0.03(0.25)			0.31(0.12)			0.05(0.17)	
Africa	p(N)	(177)	0.24(206)	0.86(55)	0.49	(177)	0.30(261)	0.30	(418)	0.26(11)	[j]	0.15	(418)	0.26(11)	0.26	(281)	0.025(143)	0.025
	β(SE)		0.21(0.18)	0.05(0.27)			0.17(0.17)			0.59(0.53)	0.26			0.59(0.53)			-0.40(0.18)	

[a] Adjusted for age, gender and Hg storage in the "low" and "high" groups, and for age, gender and exposure subgroup in the "All", "Asia" and "Africa" groups. Genotype, gender, exposure subgroup and Hg storage were modeled as categorical (nominal) variables, while age was modeled as a continuous variable.

[b] Natural ln transformed.

[c] Unstandardised β-coefficients derived from the comparison with the reference genotype, i.e., the estimated difference in ln-transformed U-Hg for two genotypes, as well as p-values and SE for these comparisons.

[d] P-value for the genotype term in the model.

[e] N = number of individuals.

[f] Control subgroups are not shown separately, but are included in the "All", "Asia" and "Africa" groups.

[g] Reference genotype.

[h] Rs2273697 *AA+AG* genotypes were pooled in all populations due to a low number of individuals with *AA* genotype.

[i] Denotes that no analyses were run, due to a low number of individuals (3 or less) for all genotypes but one.

[j] Denotes that the genotype is pooled with the heterozygotes, due to a low number of individuals (3 or less).

Table 4. Multivariate analyses[a] for *LAT1, OAT1* and *OAT3* SNPs on urinary mercury concentrations[b]: β-coefficients for each genotype with corresponding p-value and standard error (SE)[c], and model p-value[d].

Country /continent		*LAT1* rs33916661				*OAT1* rs4149170				*OAT3* rs4149182			
Exposure subgroup[f]	p(N)[e] β(SE)	*GG*[g]	*GA*	*AA*	p mod[d]	*GG*[g]	*GA*	*AA*	p mod[d]	*GG*[g]	*CG*	*CC*	p mod[d]
Indonesia													
Low	p(N)	(6)	0.41(40)	0.61(60)	0.63	(64)	0.10(35)	0.031(7)	0.050	(56)	0.15(42)	0.97(8)	0.33
	β(SE)		-0.41(0.50)	-0.24(0.48)			-0.39(0.24)	-0.97(0.45)			0.34(0.24)	-0.02(0.43)	
High	p(N)	(10)	0.68(61)	0.67(125)	0.20	(135)	0.68(57)	0.98(9)	0.92	(125)	0.60(64)	0.19(10)	0.33
	β(SE)		-0.20(0.49)	0.20(0.48)			-0.10(0.23)	-0.01(0.50)			-0.12(0.22)	0.62(0.48)	
All	p(N)	(16)	0.37(106)	0.91(199)	0.19	(215)	0.31(93)	0.40(18)	0.46	(190)	0.77(116)	0.41(19)	0.70
	β(SE)		-0.32(0.35)	-0.04(0.34)			-0.17(0.16)	-0.27(0.32)			0.05(0.16)	0.26(0.32)	
Philippines													
Low	p(N)	(0)	(33)	(80)	[h]	(92)	0.60(18)	[i]	0.60	(76)	0.17(30)	0.29(6)	0.18
	β(SE)						-0.086(0.16)				-0.28(0.20)	0.44(0.41)	
High	p(N)	(4)	0.84(30)	0.77(53)	0.94	(65)	0.85(25)	0.46(c)	0.72	(52)	0.050(35)	0.63(4)	0.14
	β(SE)		-0.13(0.66)	-0.19(0.64)			-0.06(0.31)	-0.70(0.93)			-0.54(0.27)	-0.31(0.65)	
All	p(N)	(4)	0.80(77)	0.74(158)	0.91	(186)	0.72(49)	0.47(4)	0.73	(150)	0.028(81)	0.93(11)	0.081
	β(SE)		-0.14(0.55)	-0.18(0.54)			-0.06(0.17)	-0.39(0.54)			-0.32(0.15)	0.03(0.33)	
Tanzania													
Low	p(N)	(5)	0.57(19)	0.76(28)	0.31	(12)	0.010(24)	0.022(16)	0.025	(11)	0.004(23)	0.002(17)	0.004
	β(SE)		-0.28(0.48)	0.15(0.49)			-0.81(0.30)	-0.79(0.33)			-0.91(0.30)	-1.05(0.32)	
High	p(N)	(18)	0.060(59)	0.22(58)	0.16	(48)	0.49(64)	0.039(25)	0.024	(46)	0.85(66)	0.046(25)	0.059
	β(SE)		-0.71(0.37)	-0.46(0.37)			0.18(0.27)	-0.70(0.34)			0.05(0.27)	-0.69(0.34)	
All	p(N)	(26)	0.004(93)	0.064(100)	0.015	(72)	0.79(102)	0.024(47)	0.023	(67)	0.81(105)	0.010(48)	0.017
	β(SE)		-0.79(0.28)	-0.51(0.27)			0.05(0.19)	-0.54(0.24)			-0.05(0.19)	-0.62(0.24)	
Zimbabwe													
Low	p(N)	(3)	(7)	(13)	[h]	(6)	0.20(14)	0.27(4)	0.074	(6)	0.46(14)	0.19(4)	0.13
	β(SE)						0.83(0.62)	-0.84(0.73)			0.49(0.64)	-1.04(0.76)	
High	p(N)	(25)	0.90(62)	0.51(58)	0.71	(44)	0.59(65)	0.81(37)	0.72	(43)	0.60(64)	0.70(39)	0.64
	β(SE)		-0.04(0.30)	-0.20(0.30)			0.13(0.25)	-0.07(0.28)			0.13(0.25)	-0.11(0.28)	
All	p(N)	(31)	0.61(87)	0.37(94)	0.65	(64)	0.57(100)	0.28(51)	0.22	(63)	0.72(99)	0.20(53)	0.24
	β(SE)		-0.14(0.27)	-0.24(0.26)			0.12(0.20)	-0.26(0.24)			0.07(0.21)	-0.30(0.24)	

Table 4 (continued)

Country /continent		*LAT1* rs33916661				*OAT1* rs4149170				*OAT3* rs4149182			
Exposure subgroup[f]	p(N)[e] β(SE)	*GG*[g]	*GA*	*AA*	p mod[d]	*GG*[g]	*GA*	*AA*	p mod[d]	*GG*[g]	*CG*	*CC*	p mod[d]
Asia	p(N)	(20)	0.25(183)	0.32(359)	0.51	(203)	0.88(142)	0.32(22)	0.6	(341)	0.48(198)	0.48(30)	0.55
	β(SE)		-0.34(0.30)	-0.29(0.29)			-0.02(0.12)	-0.28(0.28)			-0.08(0.11)	0.17(0.24)	
Africa	p(N)	(57)	0.017(181)	0.024(198)	0.046	(136)	0.34(207)	0.28(98)	0.12	(230)	0.66(209)	0.20(101)	0.19
	β(SE)		-0.63(0.26)	-0.59(0.26)			0.18(0.19)	-0.25(0.23)			0.09(0.19)	-0.29(0.23)	

[a] Adjusted for age, gender and Hg storage in the "low" and "high" groups, and for age, gender and exposure subgroup in the "All", "Asia" and "Africa" groups. Genotype, gender, exposure subgroup and Hg storage were modeled as categorical (nominal) variables, while age was modeled as a continuous variable.

[b] (μg/g creatinine), natural ln transformed.

[c] Unstandardised β-coefficients derived from the comparison with the reference genotype, i.e., the estimated difference in ln-transformed U-Hg for two genotypes, as well as p-values and SE for these comparisons.

[d] P-value for the genotype term in the model.

[e] N = number of individuals.

[f] Control subgroups are not shown separately, but are included in the "All", "Asia" and "Africa" groups.

[g] Reference genotype.

[h] Denotes that no analyses were run, since no individuals had the reference genotype.

[i] Denotes that the genotype is pooled with the heterozygotes, due to a low number of individuals (3 or less).