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Genetic Polymorphisms are Associated with Hair, Blood, and Urine Mercury Levels in the American Dental Association (ADA) Study Participants

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Abstract

Background/Aims—Mercury (Hg) is a potent toxicant of concern to the general public. Recent studies suggest that several genes that mediate Hg metabolism are polymorphic. We hypothesize that single nucleotide polymorphisms (SNPs) in such genes may underline inter-individual differences in exposure biomarker concentrations.

Methods—Dental professionals were recruited during the American Dental Association (ADA) 2012 Annual Meeting. Samples of hair, blood, and urine were collected for quantifying Hg levels and genotyping (88 SNPs in classes relevant to Hg toxicokinetics including glutathione metabolism, selenoproteins, metallothioneins, and xenobiotic transporters). Questionnaires were administered to obtain information on demographics and sources of Hg exposure (e.g., fish consumption and use of dental amalgam). Here, we report results for 380 participants with complete genotype and Hg biomarker datasets. ANOVA and linear regressions were used for statistical analysis.

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ETHICS

Institutional Review Board (IRB) approval was obtained from the University of Michigan (HUM00068339) and the American Dental Association.

No conflict of interest is declared.

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Results—Mean (geometric) Hg levels in hair (hHg), blood (bHg), urine (uHg), and the average estimated Hg intake from fish were 0.62µg/g, 3.75µg/L, 1.32µg/L, and 0.12µg/kg body weight/day, respectively. Out of 88 SNPs successfully genotyped, Hg biomarker levels differed by genotype for 25 SNPs, one of which remained significant following Bonferroni correction in ANOVA.

When the associations between sources of Hg exposure and SNPs were analyzed with respect to Hg biomarker concentrations, 38 SNPs had significant main effects and/or gene-Hg exposure source interactions. Twenty-five, 23, and four SNPs showed significant main effects and/or interactions for hHg, bHg, and uHg levels, respectively ($p < 0.05$), and six SNPs (in *GCLC*, *MTIM*, *MT4*, *ATP7B*, and *BDNF*) remained significant following Bonferroni correction.

Conclusion—The findings suggest that polymorphisms in environmentally-responsive genes can influence Hg biomarker levels. Hence, consideration of such gene-environment factors may improve the ability to assess the health risks of Hg more precisely.

Keywords

Single nucleotide polymorphisms (SNPs); gene-environment interaction; genetics; mercury; methylmercury; biomarker; hair; blood; urine; fish consumption; dental professionals; exposure assessment

1 INTRODUCTION

Approximately 6,600 tons of mercury (Hg) are released into the atmosphere annually, and concentrations continue to rise in many regions of the world [1]. Humans are primarily exposed to environmental Hg via ingestion of methylmercury-contaminated seafood [2]. In addition to being exposed to methylmercury via diet, the general public and dental professionals are also exposed to elemental Hg via dental amalgams [3]. Relevant exposures, especially to methylmercury, are associated with a range of clinical and sub-clinical health effects [4] thus necessitating continued monitoring efforts to gauge public health risks.

A major challenge in the risk assessment of Hg is the large inter-individual variation that has been observed in hair Hg levels, representative primarily of methylmercury exposure, after exposures of similar magnitudes in several populations [5]. Although variation in sources and dose of exposure contribute to the overall inter-individual variation in Hg biomarker levels, biochemical and physiological differences in Hg absorption, distribution, and elimination (collectively, toxicokinetics) may also play an important role. Mercury toxicokinetics may be influenced by changes, for example, in Hg binding by functional enzymes and proteins that transport, oxidize, and reduce Hg in humans [6].

Inter-individual variation in biomarker levels may have an important genetic component. For example, a twin study measuring erythrocyte Hg revealed the importance of genetic factors in Hg body burden variability [7]. A handful of epidemiological studies have now been performed to increase understanding of the genetic component of inter-individual variability in Hg accumulation, and these have mainly focused on small panels of polymorphisms in pathways known to be important for Hg toxicokinetics (glutathione pathway, selenoproteins, xenobiotic transporters, and metallothioneins) and found associations that varied by study population and Hg biomarker (see review by us [8]). As an example of early genetic studies

focused on methylmercury biomarkers, SNPs in *GSTP1* (rs1695, rs1138272) and *GCLM* (rs41303970) were associated with altered erythrocyte Hg levels adjusted for polyunsaturated fatty acids (a proxy for fish consumption) [9]. The *GCLM* SNP was also associated with higher blood, plasma, and urine Hg levels among gold miners reflective of their occupational elemental Hg exposure [10]. Recent studies continue to show the importance of glutathione pathway polymorphisms in methylmercury toxicokinetics [11, 12]. For example, several polymorphisms in this pathway were associated with differences in total Hg, speciated methylmercury levels in plasma and whole blood, and the plasma Hg to blood Hg ratio after adjusting for fish consumption [12]. Taken together, these studies point to dozens of genes that may contribute to variation in Hg toxicokinetics and potentially toxicity, though the relevance of these SNPs and other unexplored loci in related pathways to handling low-level exposures to both methylmercury and elemental Hg is not yet fully established.

The present study utilizes a population of dental professionals as we have described elsewhere [13] recruited from an American Dental Association (ADA) meeting with well-characterized exposures to both methylmercury from fish consumption and elemental Hg from dental amalgams (both in their own mouths and through occupational practices). Through this study population, we investigate the association of 88 SNPs with Hg biomarker levels representative of methylmercury (hair, blood) and elemental Hg (urine) exposures [13]. The SNPs were carefully selected from pathways and classes relevant to Hg detoxification and toxicity such as glutathione metabolism, selenoproteins, metallothioneins, hemoglobin, oxidative stress response, and xenobiotic transporters including SNPs examined in previous Hg-gene studies. Overall, we sought to validate previous findings and to also identify novel SNPs that contribute to methylmercury and elemental Hg accumulation in a population with ample data on exposure sources and three biomarkers of exposure. Identifying susceptibility factors for Hg is particularly warranted now given the United Nations Minamata Convention and the emphasis it places on public health and vulnerable populations.

2 METHODS

2.1 Study sample

In October 2012, dental professionals (including dentists, dental hygienists or assistants, dental students, and office managers) attending the Health Screening Program at the ADA Annual Session in San Francisco, California were recruited to participate in this study. All participants were informed of the study objectives and procedures. Nine hundred five participants signed a letter of informed consent and participated. Individuals were allowed to participate even if they did not complete all study procedures. Data was collected from 905 individuals, though only 630 subjects provided at least one biomarker (urine, hair, blood) for Hg analysis and 442 provided data on fish consumption patterns. From these individuals, a subset of 380 were selected for genotyping based on the following criteria: complete Hg biomarker dataset (hair, blood, urine), data on age and sex, and availability of genomic DNA sample with adequate concentration. The convenience sampling approach could have resulted in recruitment of a non-representative group of dentists, but dental professional did

not know their Hg levels or genotype before participating. Institutional Review Board (IRB) approval was obtained from the University of Michigan (HUM00068339) and the ADA.

2.2 Mercury exposure biomarker levels

Bio-specimens were collected for laboratory analyses, and complete details on sample collection and Hg analysis have been previously described by us [13]. Trained phlebotomists collected blood samples in BD Vacutainer tubes certified for trace metals analysis. Red blood cell count (RBC) was obtained with an additional fresh whole blood sample from each participant as part of a standard complete blood count (CBC) procedure. Hair was collected from the occipital region of the scalp [14]. Hair was washed with acetone and dried overnight prior to analysis. Single void spot urine samples were collected in metals-free containers and stored at 4°C until Hg analysis.

Total Hg concentrations in hair and whole blood samples (hHg and bHg) were quantified with a Direct Mercury Analyzer (DMA-80, Milestone Inc., Shelton, CT) as previously described [13]. Briefly, 0.4 mL of whole blood or 5–10 mg of 2 cm length hair from the proximal end was used for each sample. Analytical detection limits (three times the SD of blanks) were 0.09 ng for hair and 0.23 µg/L for blood. Every ten samples included quality control measures [procedural blanks, a sample replicate, one of three certified reference materials (CRMs; NIES CRM #13 for hair, INSPQ QMEQAS for blood, or DOLT-4 dogfish liver)]. Mean recovery ($\pm$ SD) of the CRMs ranged from 95.6 $\pm$ 6.7% to 100 $\pm$ 5.9%. CRM replicates had good within-day (averages for the 3 CRMs ranged from 1.8–8.0% CV) and between-day (5.3–10.3% CV) agreement. Replicate ADA hair and blood samples averaged 9.3% CV and 7.9% CV, respectively.

Total Hg in urine (uHg) was measured at the American Dental Association (ADA) laboratory via cold vapor atomic absorption spectroscopy using 2 mL of urine [15, 16]. All samples were above the analytical detection limit (0.025 µg/L). Spot urine samples were collected even though 24-hour urine collection is the gold-standard. Thus, specific gravity of urine samples was measured via refractometry (PAL-10S, Atago U.S.A., Inc., WA). Urine Hg values were adjusted by average specific gravity as we previously described [13] to decrease variability due to dilution associated with spot urine sampling [17, 18]. Statistical analyses were run with raw urine Hg values and specific gravity adjusted values as indicated.

2.3 SNP selection and genotyping

One hundred nineteen SNPs were selected that were hypothesized to underline inter-individual differences in Hg toxicokinetics and/or toxicodynamics including SNPs in genes of the following pathways and classes: glutathione metabolism, selenoproteins, metallothioneins, hemoglobin, oxidative stress response, and transporters. SNPs in genes involved in folate synthesis and DNA methylation were also included along with several SNPs previously associated with Hg toxicity (e.g., in *BDNF* and *CPOX*). DNA was isolated from 1 mL whole blood with the FlexiGene kit (Qiagen, Valencia, CA) according to the manufacturer's protocol.

Genotyping was performed using the Sequenom iPLEX Gold platform at the University of Michigan DNA Sequencing Core according to a published protocol [19]. Three multiplex

reactions (39–40 SNPs each) were run on 380 samples with four negative controls included in each batch.

2.4 Questionnaire for demographic, occupational and life style variables

Details on demographics (e.g., age, sex, and race) and smoking status were collected via self-administered questionnaires. Body mass index (BMI) was calculated (kg/m^2) from height and weight measurements taken by trained health professionals. Sources of exposure to elemental Hg were evaluated [e.g., number of personal amalgams, number of amalgams placed/removed ('handled') per week, hours worked per week, years in dental practice]. Subjects reported fish consumption patterns over the past three months (consumption frequency, portion size, and species). This data was used to estimate daily fish methylmercury intake ($\mu\text{g/kg}$ body weight/day), calculated as previously described [13, 20].

2.5 Statistical Analyses

First, distributions of all the variables were examined for normality. Mercury biomarker data and estimated Hg from fish consumption were log-transformed to achieve normality, and the log-transformed values were used for analyses unless otherwise indicated. Implausible outliers for amalgam-related variables that were previously found to be highly influential in uHg models were removed [13] which included three participants placing and/or removing >200 amalgams per week and one subject with 40 amalgams.

Out of 119 SNPs genotyped, 31 SNPs did not meet screening criteria [i.e., 5 SNPs had minor allele frequency (MAF) <0.05, 8 SNPs had call rate <80%, 3 SNPs reported a single allele only, and 15 SNPs did not achieve Hardy-Weinberg Equilibrium, HWE]. Hence 88 SNPs were considered for further analysis.

We performed T-tests to compare Hg biomarker levels and other variables of interest between participants with genetic data *vs.* those without. We performed ANOVA to examine overall group differences of log-transformed Hg biomarker levels by 3 genotypes (major homozygous, heterozygous, and minor homozygous) for 88 SNPs. Welch's ANOVA was used instead if variances were not homogenous according to Levene's test. To see the actual difference between genotype groups, TUKEYS posthoc test was employed.

The association of genotype with 3 Hg biomarkers (hHg, bHg, and uHg levels) was assessed in linear regression with each SNP coded as one variable assuming additivity of variant alleles (1= major homozygote, 2=heterozygote, 3=minor homozygote). If a SNP had <10 individuals in the minor homozygote category, heterozygotes and minor homozygotes were combined to compare no variant alleles *vs.* at least one variant allele for those particular SNPs. To determine the association between SNPs and biomarker levels while accounting for sources of exposure to elemental Hg and methylmercury, SNPs were added into linear regression models developed to predict Hg biomarker levels. Selection of the base models for hHg, bHg, and uHg levels was previously detailed [13]. In brief, models achieving the highest adjusted R square were selected from a background elimination method using variables hypothesized to influence Hg exposure biomarker levels (i.e., amalgams, Hg intake from fish consumption, occupation, sex, age, etc.). The best models (i.e., the combination of the variables without genetic variables) that explained the highest variation (21%, 14%, and

19% of hHg, bHg, and uHg, respectively) were regarded as the —base model— for the respective biomarker. The final —base models— for hHg and bHg consisted of age and Hg intake from fish, and the bHg model also included RBCs. The final predictor model for uHg included personal amalgams, amalgams handled, total years in dental practice, hours worked per week, and sex [13]. After establishing these base models for hHg, bHg, and uHg, genotype data for each of the 88 SNPs was entered into the base model one at a time along with its interaction with the primary exposure contributor (e.g., fish Hg for hHg or bHg and amalgam for uHg). Data points were excluded pairwise in the analysis since some subjects had missing data points for certain variables. Since statistical tests were run with both unadjusted and specific gravity adjusted urine Hg, both tests results are reported here.

P-values less than 0.05 were considered statistically significant. To reduce the risk of chance findings due to multiple comparisons (88 tests per biomarker), a Bonferroni correction approach was adopted in which p-values<0.0006 are considered statistically significant [21]. Statistical analyses were performed using IBM SPSS Statistics, Version 21 (IBM Corp., Armonk, NY) [22].

3 RESULTS

3.1 Characteristics of ADA participants

Table 1 summarizes the characteristics of ADA 2012 study participants. Participating current and retired dental professionals were ages 25 to 82 years old. There was no apparent selection bias since most of demographic, occupational, nutritional, and Hg exposure variables were comparable between participants (n=380) and non-participants (i.e., 525 dental professionals without genetic analysis). Yet, senior dental professionals (i.e. with higher age and total years in dental practice) were more likely to have genetic data. The mean estimated daily methylmercury intake from fish was 0.12 µg/kg/day (range, 0–0.71 µg/kg/day), with 40% of study population intakes exceeding the current U.S. EPA reference dose of 0.1 µg/kg/day [23].

3.2 Mercury biomarker levels

Total Hg concentrations were measured in hair, blood, and urine samples. Hg levels are presented in Table 1 (geometric means, GM, with arithmetic SDs). Biomarker levels ranged from 0.01 to 7.45 µg/g for hHg, 0.2 to 25.3 µg/L for bHg, and 0.14 to 11.5 µg/L for unadjusted uHg. The GM hHg to bHg ratio was 166.1 (186) which indicates large variation from the widely used hHg:bHg conversion factor of 250:1 [24, 25].

3.3 Associations between SNPs and Hg biomarker levels

Table 2 summarizes the results of ANOVA tests comparing the mean hHg, bHg, and uHg levels among SNP genotypes. Among 88 SNPs studied (see all data in Supplemental Table 1), 25 SNPs indicated significant differences (p<0.05) by genotype for at least one Hg biomarker; 10 SNPs each for hHg and uHg levels, and 11 SNPs for bHg levels. In some cases, carrying the minor allele was associated with higher Hg levels (e.g., higher uHg levels among minor homozygotes for two *SEPNI* SNPs – rs7349185 and rs229428). In other instances, the minor allele carriers had lower Hg levels (e.g., lower bHg among variant

genotypes for rs1695 in *GSTP1*). After Bonferroni correction ($p < 0.0006$), one SNP (rs2270836 in *MTIM*) remained statistically significant. Heterozygotes and minor homozygotes of rs2270836 had 38.3% and 35.8% lower hHg levels on average compared to major homozygotes, and a similar pattern was observed in bHg levels (ANOVA $p = 0.003$).

3.4 Associations between SNPs and Hg biomarker levels in multivariable analysis

SNP main effects and interactions with the major sources of methylmercury (Hg intake from fish; hHg and bHg models) or elemental Hg (personal amalgams; uHg model) were added to each base model. In general, the inclusion of SNPs into the base models improved the model adjusted r^2 . In the best cases, the adjusted r^2 increased from 0.207 to 0.245 for hHg, 0.138 to 0.175 for bHg, and 0.225 to 0.239 for specific gravity-adjusted (SG) uHg. Below we provide further details for each of the biomarkers.

For hHg, addition of genotype into the base model with Hg source interaction (genotype $\times$ fish Hg) for 88 SNPs (see all models in Supplemental Table 2) resulted in 25 SNPs with significant main effects and/or interaction terms ($p < 0.05$, Table 3 and Figure 1). Seven SNPs only had significant main effects. Among the variant genotypes (presence of at least one minor allele), three SNPs were associated with higher accumulation of Hg in hair after adjusting for the source of exposure, and the remaining four SNPs were associated with reduced hHg. Significant interactions between genotype and fish Hg were observed in hHg models for 18 SNPs. Of these, variant genotypes for 11 SNPs were associated with higher hHg concentrations per unit of Hg intake from fish consumption, and 7 SNPs with lower hHg per intake. After Bonferroni correction, five SNPs remained significant. Of these, two metallothionein SNPs (rs2270836 in *MTIM* and rs11643815 in *MT4*) had significant main effects, along with four SNP by fish Hg interactions. The reverse was observed for two SNPs in the *ATP7B* transporter (rs732774 and rs1061472) and the rs6265 SNP in *BDNF*. Figure 2 (A to E) displays the correlation between hair Hg levels and consumed fish Hg in each genotype group for the five SNPs significant at the adjusted p-value (plotted without age adjustment).

In the bHg model adjusting for Hg intake from fish, age, and RBCs, 23 SNPs had significant main effects and/or interactions with fish Hg (see significant models in Table 4 and Figure 1 and all models in Supplemental Table 3). Out of 22 significant main effects, variant alleles of nine SNPs were associated with lower bHg concentrations, while variant alleles from the remaining 13 SNPs were associated with higher bHg. Among these, rs138528239 in *GCLC* was still significant following Bonferroni correction with each variant allele associated with a 0.15 log-unit decrease in bHg. For this SNP (rs138528239), Figure 2F depicts the correlation between blood Hg levels and estimated Hg intake from fish stratified by genotype (without adjustment for age and RBCs). Nine SNPs had significant interaction terms including six that were negative, though none of these were significant following Bonferroni correction.

In the uHg models (raw uHg values), four SNPs had significant main effects (including two that also had significant interactions with amalgam) in models adjusted for amalgam, amalgams handled in the dental office, hours worked per week, total years in practice, and sex (see Table 5 and Figure 1 for significant models and Supplemental Table 4 for all uHg

models). None of these were significant following Bonferroni correction. After adjusting urine Hg for specific gravity (bottom half of Table 5), two SNPs remained significant (rs1138272 in *GSTP1* and rs2230671 in *ABCC1*), at the $p < 0.05$ level.

4 DISCUSSION

In this study, we observe significant associations between SNPs and Hg biomarker levels (hHg, bHg, uHg) as well as modification of exposure source-biomarker relationships by SNPs (see Figure 1). Out of 88 SNPs evaluated in genes hypothesized to be involved in Hg toxicokinetics or toxicodynamics, Hg biomarker levels differed by genotype of 38 SNPs (25 for hHg, 23 for bHg, and 4 for uHg) in multivariable models adjusting for key covariates including sources of exposure to methylmercury or elemental Hg ($p < 0.05$). The SNPs were in genes from the following pathways or functional categories: 8 glutathione pathway genes (12 total SNPs), 4 selenoprotein genes, 4 additional oxidative stress related genes, 3 metallothionein genes, 8 transporter genes, 2 one-carbon metabolism and methylation pathway genes, 2 genes previously linked to Hg toxicity (*CPOX* and *BDNF*), and 1 heme-related gene. While we employed two analysis schemes (ANOVA and multivariable regression), higher priority is placed on results from the regression models and genes remaining significant after Bonferroni correction ($p < 0.0006$). Six SNPs (see Figure 2) remain significant using a Bonferroni-corrected p -value. Five were associated with hHg levels (main effect: rs2270836 in *MT1M*, rs11643815 in *MT4*; interaction with fish Hg: rs732774 and rs1061472 in *ATP7B*, rs6265 in *BDNF*) and one with bHg (main effect: rs17880189 in *GCLC*).

Our study adds to the growing body of literature on genetic factors influencing susceptibility to Hg accumulation and toxicity (see review [8] and [11, 12, 26]). We addressed our hypotheses in a unique population of dentists with exposures to both methylmercury (from fish consumption) and elemental Hg (from occupational practices and personal amalgams) that are elevated compared to the general US adult population but overlapping. For example, the ADA population had nearly four times higher uHg than that of U.S. adults enrolled in the 2011–2012 NHANES (GM 0.35, 95% CI 0.30–0.40, $n = 1716$ [27]) but distributions overlapped considerably. The ADA population had higher hHg and bHg concentrations compared to the general U.S. population according to NHANES [27, 28], and this was largely due to differences in fish consumption patterns among our subjects as we previously detailed [13, 29]. Along with studying relevant exposure levels using three Hg biomarkers, this study also expanded upon the number of SNPs examined in key categories related to Hg toxicokinetics and toxicity including glutathione-related genes, selenoproteins, and transporters.

The most statistically robust results were from six SNPs that were associated with Hg levels in multivariable analysis following Bonferroni correction ($p < 0.0006$). Of these, evidence exists for the functional role of some with regards to Hg toxicokinetics (e.g., the *GCLC* SNP), while the role of others is unclear and may reflect unexplored toxicokinetic mechanisms, linkage disequilibrium with SNPs in other key genes, or indirect connections. Two SNPs in the copper transporting P-type ATPase (*ATP7B*) were associated with lower hHg (Figure 2C and D) and bHg per intake from fish for each variant allele. Since the SNPs

are in linkage disequilibrium (data not shown), either could be the functional SNP or both could be serving as markers of additional functional SNPs in *ATP7B*. While, to our knowledge, this gene has not previously been studied with regards to Hg toxicokinetics, the protein is known to have affinity for several metals, including Hg [30], and whether *ATP7B* can serve as a Hg transporter in humans merits future study.

A well-characterized nonsynonymous SNP (Val66Met, rs6265) in brain-derived neurotrophic factor (*BDNF*) was associated with lower hHg (Figure 2E) per unit Hg intake from fish consumption with each variant allele. A similar relationship was observed with bHg, though not significant at the corrected p-value. The potential for this functional SNP to impact neurobehavioral outcomes regardless of exposure has been previously observed [31, 32]. With respect to Hg toxicity, rs6265 and uHg were associated with reduced performance on several neurobehavioral tests and with indicators of anxiety and memory, and the combined impact of Hg and the SNP was additive for several tests in a population of male dentists and female dental assistants [33, 34]. While *BDNF* is thus important for Hg neurotoxicity, a mechanism by which *BDNF* influences Hg body burden has not been established.

Among the nominally significant results ($p < 0.05$), 12 SNPs came from 8 glutathione pathway genes out of 19 SNPs studied from this pathway. This pathway is the most widely studied to date with regards to Hg genetics in both elemental Hg and methylmercury exposed populations [9–12, 14, 35, 36]. Similar to previous studies [35, 36], we found SNPs in the glutamate-cysteine ligase catalytic subunit (*GCLC*, involved in glutathione synthesis) to be associated with altered bHg levels including the previously studied promoter SNP rs17883901 known to decrease gene expression and a AGCC deletion polymorphism in the 3' untranslated region (UTR; rs138528239) not studied in the past with regards to Hg biomarkers. We additionally observed biomarker differences (namely lower bHg levels) in two widely-studied glutathione s-transferase pi 1 (*GSTP1*) SNPs (rs1138272, rs1695) that agreed with results of some previous studies [9, 14] but not others [35, 36]. The impact of these SNPs, previously shown to influence gene function and interaction with exposures to metals *in vitro* [37], on Hg toxicokinetics may depend on the species of Hg, exposure dose and duration, or interaction with other genetic factors. We noted six SNPs in additional GSTs to be associated with hHg concentrations (Table 3, Figure 1), solidifying this category of genes as important to inter-individual variability in Hg toxicokinetics, specifically methylmercury. While it is known that cellular efflux of methylmercury via transporters (e.g., *ABCC1*, *ABCC2*) and ultimately biliary secretion requires glutathione conjugation, the direct role of GSTs in this process has been debated as conjugation can occur spontaneously [38]. If GSTs are not acting directly as conjugation catalysts for Hg, they may indirectly impact Hg toxicokinetics (e.g., by influencing substrate availability or by carrying Hg-glutathione conjugates).

Metallothioneins (MTs) are known to bind and sequester metals including Hg [39], and polymorphisms in MTs have previously been associated with Hg biomarker levels [36, 40]. In the ADA population, three MT SNPs out of 12 genotyped were associated with altered levels of hHg and the relationship was also reflected in bHg for two of these. The association for the *MT4* rs11643815 involved a negative main effect but positive interaction term (e.g.,

more accumulation in hHg per Hg intake from fish), and the direction of this interaction was consistent with higher hHg observed by Gundacker *et al.* with variant genotype at this SNP [36]. A similar trend of lower Hg with the variant allele of a different *MT1MSNP* (rs2270837) was observed in a study involving dental professionals from the state of Michigan [40], though the Michigan study observed this trend in uHg levels as opposed to hHg and bHg levels in the current study. The impact of these SNPs on MT expression and function merits future study.

Despite the importance of selenoproteins in Hg binding and distribution and protection against oxidative stress induced by both major species of Hg [41], to our knowledge this is only the second study to investigate selenoprotein SNPs and their relationship with Hg biomarker levels. In the study of dental professionals from Michigan, rs7579, a 3'UTR SNP in *SEPP1*, was associated with lower hHg and higher uHg per unit of estimated exposure to methylmercury and elemental Hg, respectively [14]. In the ADA population, out of 11 SNPs genotyped in 8 selenoproteins, 4 SNPs were associated with altered concentrations of at least one Hg biomarker in multivariable models (Figure 1). These included SNPs in *TXNRD2*, *SEPN1*, *SELS*, and the previously studied *SEPP1* SNP. Future gene-Hg studies should consider more SNPs in this important class and explore the mechanism of these particular proteins in Hg toxicokinetics.

This study highlights several important considerations when aiming to elucidate genetic factors underlying inter-individual variability in distribution, metabolism, and elimination of toxicants. First, multivariable analyses containing detailed information on sources of exposure are necessary. In this study, 15 of the 25 SNPs associated with Hg biomarker levels according to ANOVA ($p < 0.05$) remained significant after adjusting for sources of exposure and other covariates influencing biomarker levels. Without this adjustment, SNPs could appear significant due to random differences in actual exposure (e.g., fish consumption patterns) between genotype groups. The multivariable analyses were also able to identify 23 additional SNPs associated with Hg biomarker levels. Second, biomarker selection may influence results. Whole blood and hair are well characterized and widely used biomarkers for methylmercury exposure [42], and this study assessed the relationships between SNPs and both hHg and bHg. For many SNPs, the direction of effect was the same for the two biomarkers, though only 11 SNPs had significant ($p < 0.05$) main effects and/or interactions in both hHg and bHg models (Figure 1). Furthermore, five Bonferroni-corrected significant SNPs were observed in hHg models compared with one associated with bHg. Hair models (with or without SNPs) were better at explaining variation in hHg compared with blood models (e.g., higher adjusted r^2 values). Differences in Hg partitioning to hair versus blood and the half-life of Hg in each biomarker may lead to differences in the ability to detect Hg-gene interactions. Hair incorporates Hg as it grows, and thus the 2 cm used for analysis is expected to represent the past 2–3 months of exposure [43–45]. By contrast, blood Hg is prone to spikes following fish meals among individuals who do not regularly consume fish [46], and blood may also contain inorganic Hg [47]. Participants were not asked if they had consumed fish specifically within the last 24 hours before phlebotomy. Since a recent fish meal could have a large influence on bHg levels, the results regarding bHg should be interpreted carefully considering this potential source of error. While uHg is typically considered a biomarker of elemental Hg exposure [48], it can also contain demethylated Hg

and reflect fish consumption exposure especially in cases when sources of exposure to elemental Hg are minimal [13, 49].

Identifying SNPs associated with hHg levels may be useful to understanding not only Hg elimination via hair but also target organ deposition. The mechanisms by which methylmercury leaves the plasma and enters keratinocytes is thought to be similar to that of methylmercury's movement into target organs such as the brain [45]. Methylmercury-cysteine conjugates cross cell membranes via neutral amino acid transporters by mimicking the molecular structure of methionine [50]. In this study, transporter SNPs including some outside of the neutral amino acid carrier class were significantly associated with hHg levels ($p < 0.05$), and these genes may play a previously undiscovered role in cellular uptake and efflux of methylmercury.

This study has many strengths for addressing gene-Hg questions including data from three biomarkers (hair, whole blood, urine) for two species of Hg exposure and details on exposure sources from occupational practices and personal dental amalgams (elemental Hg) and fish consumption of 27 fish species (methylmercury). Eighty-eight SNPs in pathways important to Hg toxicokinetics and/or toxicity were successfully genotyped which included previously assessed SNPs (see review [8]) and unexplored SNPs in hypothesized pathways. This study was limited by the modest sample size ($n=380$) and the relatively large number of tests (88 SNPs for each biomarker). Even so, relationships between 38 SNPs and Hg were observed with a p -value of 0.05 that can be considered in future studies and 6 SNPs were significant after correcting for multiple testing. Due to the sample size, multiple SNPs were not analyzed together. Even so, additive effects and interactions between multiple SNPs are expected to contribute to inter-individual variability in Hg toxicokinetics. While this study was cross-sectional, Hg biomarker levels represent reported sources of exposure which were based on occupational practices common in the past year and fish consumption of the past three months, and genotype does not change with time.

5 Conclusion

The findings build upon those of previous studies to identify polymorphisms in environmentally-responsive genes that can influence Hg biomarker levels. Results suggest 38 SNPs may influence Hg toxicokinetics in glutathione metabolism, selenoprotein, metallothionein, and transporter genes along with genes from several other functional groups. Of the six SNPs significant following Bonferroni correction, variant alleles in rs6265 (*BDNF*), rs732774 and rs1061472 (*ATP7B*) are associated with lower hHg per unit intake from fish consumption. The variant allele of a *GCLC* SNP, rs138528239, is associated with lower bHg concentrations overall. In contrast, variant alleles of rs2270836 (*MT1M*) and rs11643815 (*MT4*) have negative main effects on hHg but higher accumulation in hair per unit of Hg consumption from fish.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Category	Gene symbol	SNP	Hair Hg		Blood Hg		Urine Hg	
			Main Effect	Genotype * Fish Hg	Main Effect	Genotype * Fish Hg	Main Effect	Genotype * Amalgam
Glutathione	<i>GCLC</i>	rs17883901						
	<i>GCLC</i>	rs138528239						
	<i>GGTLC1</i>	rs395485						
	<i>GLRX2</i>	rs912071						
	<i>GSTA4</i>	rs367836						
	<i>GSTA4</i>	rs405729						
	<i>GSTM1</i>	rs1065411						
	<i>GSTM3</i>	rs7483						
	<i>GSTM3</i>	rs1332018						
	<i>GSTO1</i>	rs4925						
Selenoprotein	<i>SEPNI</i>	rs2294228						
	<i>SEPP1</i>	rs7579						
	<i>SELS</i>	rs7178239						
	<i>TXNRD2</i>	rs5748469						
Oxidative Stress	<i>CAT</i>	rs7943316						
	<i>CBS</i>	rs1051319						
	<i>NOS1</i>	rs2682826						
	<i>PRDX6</i>	rs33942654						
Metallothionein	<i>MT1M</i>	rs2270836						
	<i>MT2A</i>	rs10636						
	<i>MT4</i>	rs11643815						
Transporter	<i>ABCB1</i>	rs9282564						
	<i>ABCC1</i>	rs8187858						
	<i>ABCC1</i>	rs2230671						
	<i>ATP7B</i>	rs732774						
	<i>ATP7B</i>	rs1061472						
	<i>SLC3A2</i>	rs2269353						
	<i>SLC7A7</i>	rs2281677						
	<i>SLC22A6</i>	rs4149170						
	<i>SLC22A8</i>	rs4149182						
Methylation/ Folate Pathway	<i>DNMT1</i>	rs2228613						
	<i>MTRR</i>	rs1801394						
Hg Toxicity Related	<i>BDNF</i>	rs6265						
	<i>CPOX</i>	rs1131857						
Heme	<i>HMOX1</i>	rs2071747						

LEGEND:		Positive Beta	Negative Beta
Not significant	p≥0.05		
Nominal	p<0.05		
More Significant	p≤0.001		
Bonferonni			
Corrected Sig.	p<0.0006		

Figure 1. Significant Associations between Hg Biomarker Levels and Genotype (p<0.05) from Multivariable Models

The direction and significance level are depicted for all SNPs with significant main effects and/or interaction terms in multivariable models for at least one Hg biomarker. Models adjusted for additional predicts (hHg: age, estimated Hg intake from fish; bHg: age, estimated Hg intake from fish, RBCs; uHg: total years in dental practice, amalgams, amalgams handled, hours worked per week, and sex).

Figure 2. Correlations between Hair and Blood Hg and Estimated Hg Intake from Fish Consumption Differ by Genotype

Relationships between log-transformed hHg (A–E) or bHg (F) and log-transformed Hg intake from fish consumption stratified by genotype at six SNPs significantly associated with the Hg biomarker level at a significance level adjusted for multiple testing ($p < 0.0006$) are depicted. The lines show the bivariate correlation and do not adjust for other factors included in the models. Heterozygotes and minor homozygotes were combined for *MT4* rs11643815.

Table 1

Characteristic features of the American Dental Association (ADA) study population

Demographic	Participants with genetic data		Participants without genetic data (not included)*	
	(n)	Mean (SD) or %	(n)	Mean (SD) or %
Age (years)	380	54.84 (11.36) [‡]	487	52.52 (12.55) [‡]
Male (%)	380	62.6	486	56.1
Dentist (vs Non dentist)	380	89.7	486	80.1
Red blood cell (RBC) count (x 10 ⁶ /μL)	376	4.81 (0.43)	478	4.83 (0.45)
Hg Exposure Biomarkers and Sources				
Hair Hg (μg/g) [#]	380	0.62 (1.01)	44	0.70 (0.63)
Blood Hg (μg/L) [#]	380	3.75 (3.96)	54	4.49 (3.93)
Urine Hg (μg/L) [#]	380	1.32 (1.76)	226	1.77 (1.76)
Hair: blood Hg ratio [#]	380	166.1 (186.43)	32	149.4 (362.5)
Estimated average Hg intake from fish (μg/kg/day)	366	0.12 (0.15)	48	0.08 (0.13)
Amalgams placed/assisted per week	329	5.02 (9.38)	421	5.16 (10.51)
Amalgams removed/assisted per week	329	8.41 (10.87)	421	7.08 (10.86)
Amalgams handled per week	329	13.44 (17.24)	421	12.23 (18.50)
Total years in dental practice	343	26.43 (11.31) [‡]	458	23.20 (12.02) [‡]
Hours worked per week	329	33.57 (8.42)	427	33.03 (9.59)
Amalgams in mouth	374	3.67 (3.22)	63	3.65 (3.35)

* 525 additional ADA members were recruited but not selected for genetic analysis. Numbers vary as most of these participants had some missing data.

[#] Geometric Mean (GM) reported (with arithmetic SD) due to non-normally distributed data.[‡] p<0.01 for t test comparing participants included in genetic analysis with additional ADA participants.

Table 2

Association between genetic polymorphisms and Hg levels in hair, blood and urine. Significantly different values ($p < 0.05$) are shown in **bold**. Only genes with significant ANOVA tests are shown here and all other SNPs can be found in Supplementary Table 1.*

Category	Gene symbol	SNPs	n	MAF	Hair Hg ($\mu\text{g/g}$)			Blood Hg ($\mu\text{g/L}$) Geometric Mean + Arithmetic SD			Urine Hg ($\mu\text{g/L}$)		
					I Major	2 Heterozy	3 Minor	I Major	2 Heterozy	3 Minor	I Major	2 Heterozy	3 Minor
Glutathione	<i>GCLC</i>	rs17883901	380	0.08	0.60±0.97⁷	0.73±1.19⁷	2.92±0.41⁷	3.60±4.01⁸	4.60±3.54⁸	9.65±2.30⁸	1.31±1.72	1.34±2.06	2.21±0.86
	<i>GLRX2</i>	rs912071	376	0.37	0.52±0.88¹⁰	0.72±1.09¹⁰	0.65±1.10¹⁰	3.47±4.05	4.04±4.01	3.57±3.73	1.37±1.75	1.34±1.69	1.09±1.80
	<i>GSTA4</i>	rs367836	380	0.34	0.64±1.01	0.59±0.97	0.67±1.22	4.05±4.07¹¹	3.35±3.66¹¹	4.50±4.55¹¹	1.46±1.80	1.21±1.74	1.31±1.68
	<i>GSTP1</i>	rs1695	380	0.33	0.65±1.22	0.61±0.79	0.57±0.75	4.22±4.43⁶	3.45±3.42⁶	3.15±3.49⁶	1.23±1.93	1.38±1.65	1.48±1.48
	<i>GSTP1</i>	rs1138272	380	0.06	0.65±1.04	0.46±0.69		3.87±3.96⁹	2.91±3.99⁹		1.29±1.77	1.59±1.72	
<hr/>													
Glutathione and Selenoprotein													
	<i>GPX6</i>	rs6413428	380	0.18	0.62±1.07	0.60±0.72	0.81±1.46	3.75±4.26	3.54±3.16	5.17±3.42	1.20±1.49²⁸	1.69±2.17²⁸	1.29±2.03 ²⁸
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Selenoprotein	<i>SEPN1</i>	rs7349185	369	0.15	0.65±1.06	0.55±0.93	0.71±0.64	4.00±3.84	3.18±4.46	4.66±3.15	1.31±1.90²⁶	1.24±1.25²⁶	2.36±1.31²⁶
	<i>SEPN1</i>	rs2294228	380	0.17	0.64±0.95	0.56±1.18	0.90±0.82	3.93±3.58	3.22±4.56	4.98±5.90	1.27±1.88²⁷	1.36±1.44²⁷	2.41±1.34²⁷
	<i>SEPHS2</i>	rs1133238	379	0.08	0.64±1.03	0.51±0.85		3.84±4.00	3.25±3.58		1.38±1.76²⁵	1.03±1.73²⁵	
	<i>SEPP1</i>	rs3877899	379	0.18	0.62±1.07	0.61±0.73	0.81±1.46	3.75±4.25	3.58±3.17	5.17±3.42	1.20±1.49²⁴	1.70±2.19²⁴	1.29±2.03 ²⁴
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Selenoprotein/Oxidative Stress													
	<i>TXNRD2</i>	rs5748469	380	0.49	0.66±0.91²¹	0.54±0.99²¹	0.74±1.14²¹	3.52±3.61²²	3.41±3.94²²	4.70±4.25²²	1.53±1.93	1.25±1.60	1.22±1.79
	<i>TXNRD3</i>	rs3108755	322	0.05	0.61±1.09	0.61±0.56		3.73±4.12	3.98±3.36		1.37±1.73²³	0.93±1.31²³	
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Metallothionein	<i>MT1B</i>	rs7191779	380	0.48	0.77±1.07¹⁹	0.60±0.95¹⁹	0.53±1.05¹⁹	3.90±3.90	3.67±3.87	3.73±4.24	1.30±1.96	1.36±1.77	1.27±1.50
	<i>MT1B</i>	rs8052334	379	0.48	0.76±1.06²⁰	0.59±0.94²⁰	0.54±1.06²⁰	3.89±3.89	3.65±3.83	3.73±4.26	1.29±1.95	1.36±1.76	1.25±1.50
	<i>MT1M</i>	rs2270836	378	0.35	0.81±1.10¹⁵	0.50±0.98¹⁵	0.52±0.64¹⁵	4.39±4.00¹⁶	3.42±3.74¹⁶	2.94±4.29¹⁶	1.25±1.41	1.34±2.05	1.43±1.70
	<i>MT4</i>	rs11643815	352	0.09	0.68±0.97¹⁷	0.41±0.55¹⁷	0.27±0.42¹⁷	3.93±3.80¹⁸	3.17±2.75¹⁸	1.76±1.79¹⁸	1.37±1.77	1.33±1.93	1.19±1.32
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Transporter	<i>ABCB1</i>	rs9282564	380	0.06	0.63±1.05	0.54±0.71		3.88±4.08³⁴	2.89±2.63³⁴		1.28±1.73	1.63±1.93	
	<i>ATP7B</i>	rs1801243	377	0.48	0.72±0.88	0.59±0.94	0.61±1.27	3.80±3.75	3.74±3.80	3.76±4.53	1.31±1.76¹³	1.48±1.77¹³	1.10±1.74¹³
	<i>SLC7A7</i>	rs2281677	379	0.37	0.72±0.99³⁰	0.59±1.11³⁰	0.47±0.62³⁰	3.87±3.93	3.66±4.02	3.61±3.86	1.43±1.77	1.30±1.89	1.14±1.08
	<i>SLC22A6</i>	rs4149170	362	0.11	0.60±1.00	0.70±0.77	0.83±0.57	3.61±3.85	4.31±3.99	5.52±1.85	1.39±1.80³³	1.03±1.40³³	1.75±3.25³³
	<i>SLC22A8</i>	rs4149182	380	0.23	0.58±1.10	0.70±0.90	0.69±0.55	3.35±3.90²⁹	4.51±4.19²⁹	4.01±2.23²⁹	1.29±1.84	1.34±1.70	1.57±1.39

Category	Gene symbol	SNPs	n	MAF	Hair Hg (µg/g)			Blood Hg (µg/L) Geometric Mean + Arithmetic SD			Urine Hg (µg/L)		
					1 Major	2 Heterozy	3 Minor	1 Major	2 Heterozy	3 Minor	1 Major	2 Heterozy	3 Minor
	<i>SLC43A2</i>	rs4790732	380	0.34	0.56±0.81 ³¹	0.64±1.13 ³¹	0.85±1.15 ³¹	3.29±3.71 ³²	3.98±4.26 ³²	4.81±3.61 ³²	1.30±1.77	1.37±1.62	1.24±2.17
Methylation/Folate Pathway	<i>DNMT1</i>	rs2228613	380	0.06	0.65±1.04 ⁴	0.43±0.70 ⁴		3.89±4.01 ³⁵	2.77±3.40 ³⁵		1.33±1.80	1.26±1.36	
	<i>MTHFR</i>	rs2274976	380	0.09	0.63±1.02	0.60±0.99	0.57±0.27	3.79±4.01	3.52±3.68	4.58±5.53	1.39±1.80 ⁵	1.04±1.52 ⁵	0.78±0.97 ⁵
Hemoglobin	<i>HBS1L</i>	rs4895441	378	0.26	0.58±0.90	0.69±1.14	0.57±1.07	3.68±3.74	3.94±4.28	3.12±3.77	1.43±1.89 ¹²	1.24±1.66 ¹²	0.95±0.91 ¹²

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Major: major homozygotes,
Heterozy: heterozygote,
Minor: minor homozygote, MAF: Minor allele frequency, Hg: mercury, SD: Standard deviation, n: number of participants, SNP: single nucleotide polymorphism
Details of superscript values 3–35 are provided in the Supplementary Table 1.
For SNPs with <3 individuals in the minor homozygote category, heterozygotes and minor homozygotes were combined before calculating GMs shown here.

Table 3

Parameter estimates from models of log-transformed hair Hg levels with exposure source (estimated Hg intake from fish consumption, $\mu\text{g/kg/day}$), age (not shown), genotype, and genotype-by-fish Hg interactions. Models with significant genotype variables are reported. Beta coefficients (95% CI) are reported for each predictor variable. Statistically significant coefficients ($p < 0.05$) are in **bold**.

Category	Gene symbol	SNP	n [#]	Adj R ²	Ln Fish Hg	Genotype	P- value	Genotype * Ln Fish Hg Intake	P - value
Base model ¹	<i>GGTLC1</i>	rs395485	365	0.207	4.07 (3.19 to 4.96)	—	—	—	—
			180, 153, 43	0.218	1.48 (−0.68 to 3.65)	−0.06 (−0.14 to 0.03)	—	1.90 (0.47 to 3.33)	0.010
	<i>GLRX2</i>	rs912071	151, 172, 53	0.215	6.15 (3.29 to 9.01)	0.11 (0.02 to 0.20)	0.016	−1.10 (−2.47 to 0.27)	—
	<i>GSTA4</i>	rs367836	162, 178, 40	0.223	0.80 (−1.47 to 3.07)	−0.12 (−0.21 to −0.02)	0.014	2.26 (0.82 to 3.71)	0.002
	<i>GSTA4</i>	rs405729	138, 184, 58	0.218	1.32 (−0.91 to 3.54)	−0.09 (−0.18 to −0.01)	0.038	1.85 (0.48 to 3.22)	0.008
Glutathione	<i>GSTM1</i>	rs1065411	249, 115 [#]	0.224	−0.33 (−3.29 to 2.63)	−0.12 (−0.25 to 0.01)	—	3.74 (1.37 to 6.11)	0.002
	<i>GSTM3</i>	rs7483	133, 167, 80	0.215	7.19 (4.33 to 10.05)	0.09 (0.01 to 0.16)	0.035	−1.40 (−2.61 to −0.20)	0.022
	<i>GSTM3</i>	rs1332018	174, 153, 51	0.215	3.41 (1.15 to 5.68)	−0.09 (−0.18 to −0.01)	0.038	0.42 (−0.99 to 1.82)	—
	<i>GSTO1</i>	rs4925	201, 148, 30	0.222	0.83 (−1.55 to 3.22)	−0.13 (−0.22 to −0.03)	0.008	2.35 (0.73 to 3.98)	0.005
Selenoprotein	<i>SEPN1</i>	rs2294228	264, 104, 12	0.215	1.13 (−1.43 to 3.79)	−0.11 (−0.23 to 0.01)	—	2.46 (0.45 to 4.48)	0.017
	<i>SEPP1</i>	rs7579	196, 153, 31	0.217	1.58 (−0.54 to 3.70)	−0.08 (−0.17 to 0.01)	—	1.68 (0.38 to 2.97)	0.011
	<i>SELS</i>	rs7178239	236, 124, 20	0.214	5.85 (3.51 to 8.18)	0.12 (0.02 to 0.23)	0.021	−1.29 (−2.84 to 0.26)	—
Oxidative Stress	<i>PRDX6</i>	rs33942654	229, 131, 20	0.212	6.51 (4.02 to 9.00)	0.07 (−0.03 to 0.16)	—	−1.50 (−2.93 to −0.07)	0.040
Metallothionein	<i>MT1M</i>	rs2270836	162, 167, 49	0.235	1.77 (−0.45 to 3.99)	−0.16 (−0.25 to −0.08)	0.0000	1.42 (0.08 to 2.76)	0.039
	<i>MT2A</i>	rs10636	218, 134, 27	0.213	2.40 (−0.07 to 4.87)	−0.11 (−0.20 to −0.01)	0.031	1.19 (−0.50 to 2.89)	—
	<i>MT4</i>	rs11643815	297, 55 [#]	0.245	−0.46 (−3.84 to 2.91)	−0.36 (−0.52 to −0.19)	0.0000	3.90 (1.03 to 6.78)	0.008
Transporter	<i>ABCC1</i>	rs8187858	323, 57 [#]	0.212	−0.21 (−4.43 to 4.00)	−0.13 (−0.29 to 0.04)	—	4.06 (0.15 to 7.96)	0.042
	<i>ATP7B</i>	rs732774	112, 179, 88	0.232	9.03 (6.29 to 11.76)	0.10 (0.03 to 0.18)	0.010	−2.25 (−3.43 to −1.07)	0.0000
	<i>ATP7B</i>	rs1061472	108, 180, 91	0.229	8.89 (6.06 to 11.73)	0.09 (0.01 to 0.17)	0.023	−2.16 (−3.38 to −0.95)	0.0000
	<i>SLC3A2</i>	rs2269353	325, 54 [#]	0.221	8.40 (5.25 to 11.56)	0.23 (0.06 to 0.40)	0.010	−3.86 (−6.57 to −1.16)	0.005
	<i>SLC7A7</i>	rs2281677	150, 180, 49	0.216	1.75 (−0.76 to 4.26)	−0.12 (−0.21 to −0.03)	0.012	1.43 (−0.07 to 2.93)	—
Methylation/Folate Pathway	<i>DNMT1</i>	rs2228613	339, 41 [#]	0.228	−2.96 (−8.05 to 2.14)	−0.38 (−0.60 to −0.17)	0.001	6.73 (1.88 to 11.58)	0.007
	<i>MTRR</i>	rs1801394	105, 188, 86	0.220	1.79 (−0.66 to 4.22)	−0.10 (−0.18 to −0.06)	0.021	1.34 (−0.01 to 2.69)	—

Category	Gene symbol	SNP	n [#]	Adj R ²	Ln Fish Hg	Genotype	P-value	Genotype * Ln Fish Hg Intake	P-value
Hg Toxicity Related	<i>BDNF</i>	rs6265	199, 141, 40	0.240	8.80 (6.39 to 11.21)	0.15 (0.06 to 0.24)	0.001	-2.61 (-3.84 to -1.38)	0.0000
	<i>CPOX</i>	rs1131857	260, 104, 16	0.217	6.03 (3.54 to 8.52)	0.14 (0.03 to 0.25)	0.011	-1.34 (-2.88 to 0.20)	—
Hemoglobin	<i>HMOX1</i>	rs2071747	345, 35 [#]	0.225	11.03 (6.71 to 15.35)	0.16 (0.03 to 0.30)	0.021	-3.80 (-6.12 to -1.49)	0.001

¹Base model includes age and Hg intake from fish

n= number of major homozygotes, heterozygotes and minor homozygotes, Adj R²: Adjusted R square

[#]N was <10 for minor homozygotes so minor homozygotes and heterozygotes were combined

Shading Key for Genotype

Table 4

Parameter estimates from models of log-transformed blood Hg levels with exposure source (estimated Hg intake from fish consumption, µg/kg/day), age and red blood cell count (not shown), genotype, and genotype-by-fish Hg interactions. Models with significant genotype variables are reported. Beta coefficients (95% CI) are reported for each predictor variable. Statistically significant coefficients ($p < 0.05$) are in **bold**.

Category	Gene symbol	SNP	n [#]	Adj R ²	Ln Fish Hg	Genotype	P - Value	Genotype * Ln Fish Hg Intake	P - Value
Base model ¹			365	0.138	2.64 (1.88 to 3.39)	—	—	—	—
	<i>GCLC</i>	rs17883901	326, 54 [#]	0.143	4.15 (1.58 to 6.73)	0.16 (0.00 to 0.32)	0.047	-1.38 (-3.53 to 0.77)	—
	<i>GCLC</i>	rs138528239	111, 179, 89	0.175	-1.13 (-3.67 to 1.42)	-0.15 (-0.22 to -0.08)	0.0000	1.88 (0.70 to 3.06)	—
Glutathione	<i>GSTM1</i>	rs1065411	249, 115 [#]	0.154	-0.94 (-3.46 to 1.58)	-0.15 (-0.26 to -0.04)	0.01	2.98 (0.96 to 5.00)	0.004
	<i>GSTM3</i>	rs7483	133, 167, 80	0.151	5.52 (3.08 to 7.95)	0.09 (0.02 to 0.16)	0.010	-1.31 (-2.34 to -0.29)	0.01
	<i>GSTP1</i>	rs1695	178, 155, 47	0.147	1.693 (-0.14 to 3.52)	-0.08 (-0.15 to 0.02)	0.017	0.57 (-0.49 to 1.63)	—
Selenoprotein	<i>SEPN1</i>	rs2294228	264, 104, 12	0.155	-0.39 (-2.56 to 1.79)	0.14 (-0.25 to -0.04)	0.006	2.52 (0.81 to 4.23)	0.004
	<i>SEPP1</i>	rs7579	196, 153, 31	0.145	1.315 (-0.49 to 3.12)	-0.09 (-0.17 to -0.01)	0.024	0.89 (-0.21 to 1.99)	—
	<i>TXNRD2</i>	rs5748469	112, 167, 101	0.156	5.08 (2.69 to 7.46)	0.11 (0.04 to 0.17)	0.002	-1.09 (-2.07 to -0.11)	0.029
Oxidative Stress	<i>CAT</i>	rs7943316	131, 168, 80	0.152	4.59 (2.46 to 6.71)	0.09 (0.03 to 0.16)	0.029	-0.92 (-1.84 to 0.00)	—
	<i>NOS1</i>	rs2682826	206, 138, 35	0.143	3.68 (1.62 to 5.74)	0.08 (0.00 to 0.16)	0.04	-0.65 (-1.78 to 0.47)	—
Metallothionein	<i>MT1M</i>	rs2270836	162, 167, 49	0.152	1.74 (-0.17 to 3.65)	-0.09 (-0.17 to -0.02)	0.010	0.51 (-0.64 to 1.67)	—
Transporter	<i>ABCB1</i>	rs9282564	334, 46 [#]	0.147	0.59 (-2.28 to 3.45)	-0.19 (-0.34 to -0.04)	0.016	1.84 (-0.67 to 4.36)	—
	<i>ABCC1</i>	rs8187858	323, 57 [#]	0.145	-0.96 (-4.55 to 2.63)	-0.15 (-0.29 to -0.01)	0.036	3.36 (0.03 to 6.68)	0.048
	<i>ATP7B</i>	rs732774	112, 179, 88	0.156	5.69 (3.34 to 8.04)	0.10 (0.04 to 0.17)	0.003	-1.40 (-2.41 to -0.38)	0.007
	<i>ATP7B</i>	rs1061472	108, 180, 91	0.154	5.76 (3.34 to 8.19)	0.10 (0.03 to 0.17)	0.005	-1.41 (-2.45 to -0.38)	0.008
	<i>SLC22A8</i>	rs4149182	228, 130, 22	0.15	3.97 (2.12 to 5.82)	0.12 (0.03 to 0.20)	0.007	-0.97 (-2.16 to 0.22)	—
	<i>SLC43A2</i>	rs4790732	169, 162, 49	0.148	3.68 (1.42 to 5.94)	0.09 (0.02 to 0.16)	0.019	-0.60 (-1.71 to 0.51)	—
Methylation/Folate Pathway	<i>CBS</i>	rs1051319	334, 46 [#]	0.144	4.54 (2.25 to 6.82)	0.16 (0.01 to 0.31)	0.035	-1.50 (-3.13 to 0.13)	—
	<i>DNMT1</i>	rs2228613	339, 41 [#]	0.147	-0.42 (-4.79 to 3.96)	-0.22 (-0.40 to -0.03)	0.021	2.89 (-1.28 to 7.06)	—
	<i>MTRR</i>	rs1801394	105, 188, 86	0.145	0.70 (-1.38 to 2.78)	-0.08 (-0.15 to -0.01)	0.027	1.14 (-0.02 to 2.29)	—
Hg Toxicity Related	<i>BDNF</i>	rs6265	199, 141, 40	0.142	4.62 (2.53 to 6.72)	0.06 (-0.02 to 0.13)	—	-1.09 (-2.16 to -0.02)	0.045

Category	Gene symbol	SNP	n [#]	Adj R ²	Ln Fish Hg	Genotype	P - Value	Genotype * Ln Fish Hg Intake	P -Value
	<i>CPOX</i>	rs1131857	260, 104, 16	0.144	4.51 (2.38 to 6.65)	0.10 (0.01 to 0.19)	0.035	-1.26 (-2.58 to 0.05)	—
Hemoglobin	<i>HMOX1</i>	rs2071747	345, 35 [#]	0.158	8.14 (4.46 to 11.82)	0.18 (0.07 to 0.30)	0.002	-3.02 (-4.99 to -1.04)	0.003

/ Base model includes age, fish intake, and RBC count

n= number of major homozygotes, heterozygotes and minor homozygotes, Adj R²: Adjusted R square, RBC: red blood cells

[#]N was <10 for minor homozygotes so minor homozygotes and heterozygotes were combined

Shading Key for Genotype:

Table 5

Parameter estimates from models of log-transformed urine Hg levels with exposure sources (amalgam in mouth, working hours, total years in practice, amalgams handled), sex, genotype, and genotype-by-amalgam interactions. Models with significant genotype variables are reported. Unstandardized coefficients (95% CI) are reported for each predictor variable. Statistically significant coefficients ($p < 0.05$) are **in bold**.

Category	Gene Symbol	SNP	n [#]	Adj R ²	Amalgam	Genotype	P - Value	Genotype * Amalgam	P - Value
Base model ¹			365	0.187	0.04 (0.02 to 0.05)	—	—	—	—
Glutathione	<i>GSTP1</i>	rs1138272	336, 44 [#]	0.198	0.09 (0.04 to 0.13)	0.22 (0.02 to 0.42)	0.031	-0.05 (-0.09 to -0.01)	0.015
Transporter	<i>ABCC1</i>	rs2230671	220, 140, 20	0.195	0.07 (0.03 to 0.10)	0.12 (0.01 to 0.22)	0.028	-0.02 (-0.04 to 0.00)	0.052
	<i>SLC22A6</i>	rs4149170	290, 72 [#]	0.197	-0.00 (-0.05 to 0.04)	-0.20 (-0.36 to -0.03)	0.019	0.03 (-0.00 to 0.07)	0.058
Methylation/Folate Pathway	<i>CBS</i>	rs1051319	334, 46 [#]	0.196	-0.01 (-0.05 to 0.03)	-0.21 (-0.41 to -0.02)	0.029	0.04 (0.00 to 0.08)	0.038
Specific gravity adjusted urine									
Base model ¹			365	0.225	0.07 (0.05 to 0.09)	—	—	—	—
Glutathione	<i>GSTP1</i>	rs1138272	336, 44 [#]	0.239	0.17 (0.09 to 0.26)	0.32 (-0.05 to 0.69)	0.091	-0.09 (-0.16 to -0.02)	0.009
Transporter	<i>ABCC1</i>	rs2230671	220, 140, 20	0.232	0.12 (0.06 to 0.18)	0.20 (0.01 to 0.39)	0.035	-0.03 (-0.07 to 0.00)	0.065
	<i>SLC22A6</i>	rs4149170	290, 72	0.224	-0.03 (-0.05 to 0.11)	-0.18 (-0.48 to -0.12)	0.241	0.03 (-0.03 to 0.09)	0.316
Methylation/Folate Pathway	<i>CBS</i>	rs1051319	334, 46 [#]	0.227	0.01 (-0.07 to 0.09)	-0.28 (-0.63 to 0.08)	0.125	0.05 (-0.02 to 0.12)	0.136

¹Base model includes amalgam in mouth, gender, amalgam handled, work hours per week and total years in dental practice. Amalgam in mouth, gender, work hours per week and total years in dental practice are not shown in Table. Supplementary Table 5 has all the data from the models.

n= number of major homozygotes, heterozygotes and minor homozygotes, Adj R²: Adjusted R square

[#]N was <10 for minor homozygotes so minor homozygotes and heterozygotes were combined

Shading Key for Genotype:

p<0.0006 p<0.01 p<0.05