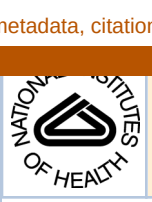

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An Investigation of Modifying Effects of Single Nucleotide Polymorphisms in Metabolism-related Genes on the Relationship between Peripheral Nerve Function and Mercury Levels in Urine and Hair

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Abstract

Mercury (Hg) is a potent neurotoxicant. We hypothesized that single nucleotide polymorphisms (SNPs) in genes coding glutathione-related proteins, selenoproteins and metallothioneins may modify the relationship of mercury biomarkers with changes in peripheral nerve function. Dental professionals (n=515) were recruited in 2009 and 2010. Sensory nerve function (onset latency, peak latency and amplitude) of the median, ulnar and sural nerves were recorded. Samples of urine, hair and DNA were collected. Covariates related to demographics, nerve function and elemental and methyl- mercury exposure were also collected. Subjects included 244 dentists (47.4%) and 269 non-dentists (52.2%; mostly dental hygienists and dental assistants). The mean mercury levels in urine (1.06μg/L) and hair (0.51μg/g) were not significantly different from the US general population (0.95 μg/L and 0.47μg/g, respectively). In multivariate linear models predicting nerve function adjusting for covariates, only 3 out of a total of 504 models showed stable and statistically significant interaction of SNPs with mercury biomarkers. Overall, given the possibility of false positives, the results suggested little evidence of effect modification of the SNPs on the relationship between mercury biomarkers with peripheral nerve function at exposure levels that are relevant to the general US population.

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Keywords

Mercury; Single Nucleotide Polymorphism; Nerve Function; Biomarker; Gene-environment Interaction; Metallothionein; Glutathione; Selenoprotein

1. Introduction

Mercury is ubiquitous in the environment. The general population has low but measurable levels of mercury in hair (mostly through fish consumption) and urine (mostly through dental amalgam). Much of the work on the peripheral neurological impact of mercury has been based on clinical neurological examinations of the peripheral nervous system at high levels of exposure (Albers et al. 1982; Albers et al. 1988; Ellingsen et al. 1993; Letz et al. 2000; Levine et al. 1982). However, clinical neurological examinations may not be adequate to detect subclinical effects of low-level exposure to methylmercury and elemental mercury. Nerve conduction tests, widely used in clinical practice and research to evaluate peripheral neuropathy, can serve as an objective measure of peripheral nerve impairment in relation to mercury at low levels.

There are four prior studies of low-level elemental mercury exposure in which clinical neurological examinations were used to associate signs and symptoms of peripheral neuropathy with elemental mercury exposures (DeRouen et al. 2006; Echeverria et al. 2005; Echeverria et al. 2006; Kingman et al. 2005). Two of these studies employed nerve conduction tests, but they focused solely on motor nerve conduction (DeRouen et al. 2006; Echeverria et al. 2005). Motor nerve conduction is generally considered a less sensitive indicator of peripheral nerve impairment than sensory nerve conduction (Shefner and Dawson. 1990). Furthermore, ulnar nerve conduction in the hand was the only measurement used in the two studies. However, the use of the sural nerve in the lower extremities is likely to be more sensitive to the effects of peripheral neurotoxicants than the ulnar or median nerves in the hand as nerves with longer axons are typically more susceptible to neurotoxicants. This has been shown in patients with most forms of neurotoxin- or neurotoxicant-induced peripheral neuropathy (Kimura, 1983). Although three of the four studies reported vibration sensitivity of toes and fingers in relation to elemental mercury exposure (Echeverria et al. 2005; Echeverria et al. 2006; Kingman et al. 2005), sensory nerve conduction is considered a more objective index of peripheral nerve impairment compared to vibrotactile measurement in the lower extremities (Kingman et al. 2005). A study that employs nerve conduction of sensory nerves (including the sural nerve) in the investigation of the association of peripheral nerve function with elemental mercury exposure is warranted. There has been no prior study of low-level methylmercury exposure in relation to the peripheral nervous system.

Combined with five other prior studies (Custodio et al. 2004; Custodio et al. 2005; Gundacker et al. 2007; Gundacker et al. 2009; Schläwicke Engström et al. 2008), our recent findings suggested that single nucleotide polymorphisms (SNPs) in metallothionein (MT) genes, key glutathione synthesizing genes (glutamate cysteine ligase (GCL)), glutathione-S-transferase genes (GST) and selenoprotein genes may modify the relationship of low-level exposure to elemental mercury and methylmercury with biomarker levels in urine and hair (Goodrich et al. 2011; Wang et al. 2011). While the underlying mechanism for such effect modification was not clear, these polymorphisms were hypothesized to influence mercury-binding capacity of the enzymes and the protein products through alternations of gene expression and enzyme activity. Furthermore, protein products of these genes all have been found to serve as anti-oxidants in humans (Aschner et al. 2006; Kumari M.V. et al. 1998; Ravn-Haren et al. 2006; Schurz et al. 2000). Anti-oxidants have been found to improve

nerve function through reduction of oxidative stress in rodents (Cameron et al. 1993; Vincent et al. 2007).

In light of all these findings and the potential subclinical impact of mercury on peripheral nerve function, we extended our investigation of effect modification of these SNPs into the relationship between nerve function and mercury levels in urine and hair. In particular, the two previous studies that did use nerve function did not find any association between biomarkers and nerve function, and they might have missed subtle effects of mercury due to (unmeasured) interactions between genetic polymorphisms and mercury biomarkers. By employing sensory nerve function and assessment of SNPs of key metallothionein, selenoprotein and glutathione-related proteins, the goal of the present study was to evaluate potential interaction between SNPs and mercury biomarker levels (both urine and hair) on nerve function at an exposure level that is relevant to the US general population.

2. Materials and Methods

Subjects were recruited during the Michigan Dental Association (MDA) annual conventions held in 2009 (n=232) and 2010 (n=283). They represent a convenience sample of dental professionals who attended the conventions. Participants provided written consent. The study was approved by the University of Michigan Institutional Review Board. Subjects completed a self-administered questionnaire to provide information about recent mercury exposures from sources of both elemental mercury and methylmercury, demographic information, history of pre-existing diseases and other covariates.

2.1 Urine and Hair Specimens

Each subject provided a spot urine sample in a mercury-free container (Becton, Dickinson, and Company; NJ, USA). A minimum of 10 mg (approximately 10–20 hair strands) of hair was collected from the occipital region of the head. We were not able to obtain urine and hair samples from 13 and 10 subjects, respectively.

Total mercury content in urine and hair (first 2cm hair closest to the scalp) samples was determined using atomic absorption spectroscopy in the Direct Mercury Analyzer-80 (DMA-80, Milestone Inc., Shelton, CT) based on US Environmental Protection Agency Method 7473 as described elsewhere (Goodrich et al. 2011). No hair or urine sample was below the Theoretical Method Detection Limit (TMDL), calculated as 3 times the standard deviation of measurement averages of all blanks (urine-2009:0.05ng; 2010: 0.01ng; hair-2009: 0.07ng; 2010: 0.01ng).

2.2 Measurements of Nerve Conduction Function

Nerve conduction function measures the electrical conductivity of a nerve in response to stimulus. Sensory nerve conduction was performed on each subject and included measurements of amplitude, onset latency and peak latency of the median and ulnar sensory nerves in the right wrist and the sural nerve in the right ankle. In general, higher amplitude and lower latency reflect better nerve function. The temperatures of the right midpalm and midfoot at the time of measurements were also recorded. The hand and/or foot was warmed with electric heating pads if the limb was initially below 32 °C.

Antidromic stimulation was applied 14cm proximal to standard ring-shaped recording electrodes, separated by a distance of 3cm and placed on digits II and V of the right upper extremity for median and ulnar sensory nerves, respectively. A TECA Synergy (Oxford Instrument, Hawthorne, NY) was used to record the amplitude, onset latency and peak latency upon the stimulation over the median and ulnar sensory nerves. For measuring nerve conduction of the sural sensory nerve, antidromic stimulation was applied on the posterior

aspect of the right calf, 14cm proximal to the recording electrode placed behind the lateral malleolus in the lower extremity. The peak and onset latencies (milliseconds-ms) were defined as the time required for an electrical stimulus to initiate the first peak of an action potential waveform and the time to deflect from baseline of waveform, respectively. The amplitude (microvolts-μV) was defined as the baseline-to-peak voltage difference on a waveform. All parameters were recorded in accordance with the guidelines outlined by the American Association of Electrodiagnostic Medicine (American Association of Electrodiagnostic Medicine. 2002).

2.3 Hand Diagrams

A standard self-administrated hand diagram was given to each participate (Katz and Stirrat. 1990; Katz et al. 1990). Subjects shaded the areas where numbness, tingling, burning or pain had occurred more than three times, or had lasted more than one week in the six months prior to the measurement. The diagrams were then reviewed and scored independently by two experienced physicians for symptoms consistent with Carpel Tunnel Syndrome (CTS). Discrepancies in the results were reconciled through consensus.

2.4 SNP Selection and Genotyping

Buccal swabs were used to collect DNA samples (Goodrich et al. 2011). Genomic DNA was isolated and purified for genotyping using Promega SV Genomic kits (Promega Corporation, Fitchburg, WI). A total of twenty-six SNPs and two deletion polymorphisms in genes important for mercury metabolism such as metallothionein, selenoprotein and glutathione-related proteins were genotyped using TaqMan Allelic Discrimination Assay (Applied Biosystems, Foster City, CA), Restriction Fragmentation Length Polymorphism (RFLP) genotyping or PCR-based deletion-detecting method (See Appendix A Table A.1).

We selected fifteen SNPs from selenoprotein and glutathione-related genes along with thirteen metallothionein SNPs (See Appendix A Table A.1). Ten of these polymorphisms (*GSTP1*-105, *GSTP1*-114, *GSTT1* deletion, *GSTM1* deletion, *GCLM* -588, *GCLC* -129, *MT1M* (rs2270836), *MT1A* (rs8052394), *MT1E* (rs708274), and *MT1M* (rs9936741)) have been previously reported to significantly modify the mercury biomarker-exposure relationship in humans (Custodio et al. 2004; Custodio et al. 2005; Gundacker et al. 2007; Gundacker et al. 2009; Schläwicke Engström et al. 2008). The eighteen remaining SNPs were chosen based on: 1) the importance of the gene category to which they belong in mercury metabolism (glutathione s-transferases, enzymes involved in glutathione synthesis, selenoproteins or metallothionein); 2) SNP minor allele frequency ≥ 5% in the Centre d'Etude du Polymorphisme Humain (CEPH) panel as reported in the PubMed SNP database; 3) a known or hypothesized effect of SNP on enzyme function or gene expression (e.g., the SNP encodes an amino acid change or a change in an important regulatory region such as the 5′UTR or 3′UTR). All selected SNPs had minor allele frequencies ≥ 5% and all met Hardy-Weinberg Equilibrium (HWE). Not all SNPs were genotyped in subjects from both sampling years (See Appendix A Table A.1)

2.5 Statistical Methods

2.5.1 Exclusion Criteria—Different exclusion criteria were applied to models depending on the specific analyses involved. We excluded subjects with self-reported conditions that have an impact on nerve function or kidney function. One subject with Charcot-Marie-Tooth (CMT) and three pregnant women were excluded from all analyses. When urinary mercury was included in the analyses, we excluded 8 subjects with preexisting kidney diseases (lithiasis, pyelonephritis, orthostatic proteinuria, end stage kidney disease or chronic renal failure). For modeling nerve conduction of ulnar and sural sensory nerves in relation to urinary mercury, we additionally excluded 24 subjects with self-reported history of stroke or

diabetes. For modeling nerve conduction of the median sensory nerve in relation to urinary mercury, we additionally excluded 48 subjects with a self-reported history of CTS or rheumatoid arthritis along with 11 additional subjects diagnosed with CTS from the hand diagram. For analyses of ulnar or median sensory nerves in relation to hair mercury, only subjects with stroke or diabetes were excluded. For the median nerve in relation to hair mercury level, the 48 additional subjects with a history of CTS or rheumatoid arthritis along with the 11 additional subjects diagnosed with CTS from the hand diagram were excluded. As a result, the final sample sizes were of 405, 474 and 465 for median, ulnar and sural nerves, respectively after excluding missing urine and hair measurements.

2.5.2 Statistical Methods—All statistical analyses were performed in SAS 9.2 (SAS Institute Inc., Cary, NC). The multivariate linear regression analyses were conducted in three phases. In the first phase, using multivariate linear regression models, each log-transformed nerve conduction measurement was regressed against covariates including race, gender, height, weight, age, occupation, dominant hand, hand temperature and foot temperature. A base model predicting each nerve measurement was then formed using the statistically significant predictors derived from the first phase of analyses. A total of nine base models were developed for amplitude, onset latency and peak latency of the median, ulnar and sural nerves (See Appendix B Table B.1). In the second phase, urine and hair mercury biomarker levels were independently added to each of the nine base models resulting in eighteen models (See Appendix B Table B.1). In the third phase of the analyses, for each SNP, the main effects and interaction terms with the mercury biomarker using dummy variables for the heterozygote and minor homozygote with the major homozygote as referent were added to the eighteen models (Appendix B Table B.2). Such interactions were investigated one SNP at a time in separate models, resulting in a total of 504 (18 × 28) separate models. The SNP-biomarker interaction was the main outcome of interest. For each of the models (12 out of 504) that achieved statistical significance in SNP-biomarker interaction terms, a sensitivity analysis was performed to identify influential observations whose urine or hair mercury levels had “large” impact on respective nerve function modeled. In each of the 12 models, a subject was considered a potentially influential observation if the absolute value of the DFBETA statistic of a biomarker-dependent variable (either urine or hair mercury biomarker) in relation to the respective modeled nerve conduction measurement was equal to/greater than the conventional cutoff value (2/sqrt(regression model sample size)). These influential observations were ranked in descending order according to the DFBETA statistics and were excluded if the Cook’s D statistic of the observations exceeded the conventional cutoff value (4/(regression model sample size)) in the sensitivity analysis. Models were labeled as “stable” if exclusion of any or all influential observations did not affect the significance or parameter estimate direction of interaction terms. Models were labeled “unstable” if such exclusion resulted in changes of parameter significance (from significant to nonsignificant) or in the direction of interaction parameter estimates (See Appendix Table B.2). The default significance level was 0.05.

3. Results

3.1 Demographics

The total sample includes 515 subjects (Table 1) and is predominantly Caucasian (90.5%). Dentists comprised 47.4% of the sample. There were 198 males (38.5%). Thirty six percent (n=184) of the subjects were male dentists while about 50% (n=255) were female non-dentists. The mean ages for dentists and non-dentists were 56.1 and 48.2 years old, respectively, and this difference was significant. The mean ages for Caucasians and non-Caucasians are 52.5 and 46.8 years old, respectively, and they also differed significantly.

The mean Body Mass Indices (BMIs) of dentists and non-dentists did not differ significantly and neither did that of Caucasians and non-Caucasians.

3.2 Levels of Urine and Hair Biomarkers

Both the geometric mean levels of urinary and hair mercury in our study are higher than that in the US general population but the distributions did not differ significantly from the US general population (see Appendix Tables A.2).

3.3 Associations between Nerve Conduction Measurements and Covariates

Table 2 shows the overall mean results of nerve conduction measurements (amplitude, onset latency and peak latency) for all three nerves tested (median, ulnar and sural). The log-transformed nerve conduction measurements of all three nerves were predicted by different sets of covariates in multivariate linear regression models (see Appendix Table B.1).

Increased age was shown to be inversely associated with measured nerve function across all nerve conduction measurements. “Better” nerve function was observed in females compared to males for all ulnar models in which gender was a significant covariate. BMI was inversely associated with nerve function in all median models. Height was inversely associated with nerve function in all sural models. Limb temperature was inversely associated with latency in all nerves and ulnar nerve amplitude but was not significantly associated with amplitude in the median or sural nerves.

3.4 Results of Stable Multivariate Regression Models Exploring Effect Modification of Selected SNPs (Adjusted for mercury levels in Urine and Hair and Covariates)

Only 3 out of 504 multivariate linear regression models of log-transformed nerve conduction measurements achieved stable, statistical significance for the SNP-biomarker interaction terms (Table 3). Compared to those with CC genotypes, subjects with CT genotypes of the *GPX1* (rs1050450) SNP had lower ulnar peak latency for the same increment of urine mercury levels. Subjects with GA genotypes of the *GCLC* 5′UTR (rs17883901) SNP had higher sural amplitude compared to those with GG genotypes for the same increment of hair mercury levels. Subjects with GT or TT genotypes of the *MT1E* 3′UTR (rs708274) had higher ulnar onset latency compared to those with GG genotypes for the same increment of hair mercury levels. A stratification analysis of respective nerve measurements for these three genotypes showed no statistically significant differences between genotypes (Table 4).

3.5 Results of Unstable Multivariate Regression Models Exploring Effect Modification of Selected SNPs (Adjusted for mercury levels in Urine and Hair and Covariates)

Nine other multivariate linear regression models (out of 504 models) of log-transformed nerve conduction measurements also achieved statistical significance for the SNP-biomarker interaction terms (See Appendix Table B.2). However, these results were unstable and did not hold up after excluding potentially influential observations.

4. Discussion

We measured sensory nerve conduction function including amplitude, onset latency and peak latency in median, ulnar and sural nerves, respectively in a dental population exposed to elemental and methyl- mercury at levels similar to the US general population. We explored whether SNPs and deletion polymorphisms in genes related to mercury metabolism may modify the association of mercury levels in urine and hair, respectively with nerve conduction measurements in multivariate linear regression models. Only 3 out of 504 multivariate models that investigated effect modification of SNPs achieved stable significance in those interaction terms (Table 3). In another 9 models, unstable significant

interaction terms were found and such effect modification did not hold after sensitivity analyses were performed on those models (See Appendix Table B.2). Given the few stable significant findings, the results provide little evidence of effect modification of the SNPs on the relationship between mercury biomarker levels and peripheral nerve function.

The median and ulnar nerve conduction values in our study were within the range of values previously reported in workers but with a mean age 16 years younger than the present study (Salerno D F et al. 1998). The sural nerve values were also within the expected range (Schuchmann. 1977). Coefficients of covariates in models predicting nerve function, such as age, gender, height, weight, BMI, and temperature, were similar in magnitude and direction to those reported in prior studies (Fujimaki et al. 2009; Greathouse et al. 1989; Kimura, 1984; Letz and Gerr. 1994; Rivner et al. 2001; Robinson et al. 1993; Salerno D F et al. 1998; Tong et al. 2004; Trojaborg et al. 1992; Werner and Franzblau. 1996; Werner. 2006).

We are not aware of any prior study that has looked at the effect of genetic polymorphisms on the associations between nerve function and mercury levels in urine or hair. Given the small number of stable and significant interaction terms (n=3) and the large number of models tested (n=504), it is not possible to rule out that the effect modification of *GPX1* (rs1050450), *GCLC* 5′UTR (rs17883901) and *MT1E* 3′UTR (rs708274) SNPs may be false positives as a result of multiple comparisons. Therefore, we conclude that there is no sufficient evidence of effect modification of these SNPs on the relationship between nerve function and mercury burden in the body. However, given the significant findings from our prior studies which indicated that some of these SNPs modify the relationship between mercury exposure and biomarker levels (Goodrich et al. 2011; Wang et al. 2011), our present null result suggests that the effect modification of these SNPs in explaining the variability of health effects in response to mercury exposure is restricted to the exposure-biomarker relationship. Thus, any observed variation of nerve function in response to mercury may be explained primarily through enzyme binding of mercury at a metabolic level, as hypothesized in our prior studies (Goodrich et al. 2011; Wang et al. 2011)..

Differential findings of the effects of the SNPs on outcome-biomarker (urine or hair) relationship are not surprising given that the binding of heavy metals depend on several factors: abundance of the enzymes/proteins in target tissues, mercury form in target tissues and redox chemistry of the enzymes/proteins. Inorganic mercury and methylmercury differ in their target organs (kidney versus brain) and elimination routes (urine versus feces). Thus, levels of enzymes/proteins vary across the target tissues, which may contribute to the differential modifications.

The study suffers from a number of limitations. First, the study has a relatively small sample size of dental professionals available for analysis. For some SNPs, e.g. *MT1M* (rs2270836), there were fewer than 15 subjects with the homozygous variant genotype. The small numbers limit the power of our study to assess effect modification of mercury metabolism related genes on outcome-biomarker relationships. Second, our study group was a convenience sample, not a random sample. However, there is no reason to believe that subjects had any prior knowledge of their genotypes or mercury levels. Nevertheless, convenience samples may have other hidden biases. For example, our subjects were predominately Caucasian, which may have introduced bias. Third, the selection of SNPs was based on their potential relationships with mercury exposures rather than adverse neurological outcomes. Without taking into account SNPs related to adverse neurological diseases, it may not be surprising to have found so few stable and significant results out of the 504 models examined. Despite the fact that nerve conduction is a sensitive and objective technique for assessing peripheral nerve impairment, variability and errors in those measurements may still contribute to the inconsistent results for sural nerve measurements

(Kimura, 1984). In our study, the sural nerve did not appear to be more susceptible to mercury compared to the ulnar or median nerves (See Appendix Table B.1). Due to the length of the sural nerve, greater variability may exist in the sural measurements compared to those for median and ulnar nerves. The results are relevant to the general population although they might not be generalizable to non-Caucasian population.

We did not correct for potential multiple comparison errors due to potential high correlations between nerve measurements ($r>0.85$ within the same nerve; $r<0.5$ within the same type of measure between nerves) in different nerves when analyzing modifying effects of SNPs on the relationship of nerve function with mercury levels in hair and urine. Since there were so few significant interaction terms showing modifying effects, we would not have drawn a conclusion that was any different from the current one had we made such correction. Any correction for multiple comparisons would have decreased the default cutoff p value ($p=0.05$) and thus resulted in even fewer or no significant results since most of the p value ranged from 0.01 to 0.05.

5. Conclusion

The study is the first to investigate the potential modifying effects of key SNPs related to mercury metabolism on the relationships between low-level mercury in both urine and hair, respectively with objective sensory nerve function measurements in median, ulnar and sural nerves. We carried out the study in a predominately white population with mercury biomarker levels relevant to the US general population. Overall, given the possibility of false positives the results suggest little evidence of effect modification of the SNPs on the relationship between mercury biomarkers and peripheral nerve function. Future research is needed to fully investigate the role of genetic polymorphisms in the development of adverse neurological outcomes related to mercury exposures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CEPH	Centre d'Etude du Polymorphisme Humain
CMT	Charcot-Marie-Tooth
CTS	Carpel Tunnel Syndrome
DMA	Direct Mercury Analyzer
GCL	Glutamate Cysteine Ligase
GPX	Glutathione Peroxidase
GSR	Glutathione Reductase

GSS	Glutathione Synthetase
GST	Glutathione-S-transferase
HWE	Hardy-Weinberg Equilibrium
MDA	Michigan Dental Association
MT	Metallothionein
NHANES	National Health and Nutrition Examination Survey
RFLP	Restriction Fragment Length Polymorphism
SEP	Selenoprotein
SNP	Single Nucleotide Polymorphism
TMDL	Theoretical Method Detection Limit

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Highlights

1. First to investigate modifying effects of SNPs on biomarker-nerve associations
2. Results are applicable to the US general population
3. No modifying effects of glutathione protein, selenoprotein and metallothionein SNPs

Table 1

Demographics

	N	Age (year;SD)	BMI (kg/m^2;SD)	Female
Occupation				
Dentist	244	56.1 (11.6)*	26.4 (4.0)	60 (24.6%)
Non-dentist	269	48.2 (11.2)*	26.4 (5.3)	255(94.8%)
Subtotal	513			315 (61.4%)
Missing	2			2
Race				
Caucasian	463	52.5 (11.9)#	26.3 (4.6)	
Non-Caucasian	49	46.8 (12.5)#	27.1 (6.1)	
Subtotal	512			
Missing	3			

*p<0.005;

#p<0.0001,

SD: standard deviation

Table 2

Mean Amplitude, Onset and Peak Latencies of Median, Ulnar and Sural Nerves

	Onset Latency (msec)			Peak Latency (msec)			Amplitude (μV)		
	Median (N)	**Ulnar (N)**	**Sural (N)**	**Median (N)**	**Ulnar (N)**	**Sural (N)**	**Median (N)**	**Ulnar (N)**	**Sural (N)**
Mean	2.65(405)	2.51(474)	3.02(465)	3.41(405)	3.24(474)	3.81(465)	29.35(405)	27.85(474)	13.12(465)

Table 3

Coefficients and p Values from Stable Multivariate Linear Regression Models (3 out of 504) of Log-transformed Nerve Conduction Function Predicted by Hg Biomarker Levels in Urine or Hair, SNP Genotypes and SNP-biomarker Interactions

			Base model															
	SNP Name	db SNP	R^2	Intercept	Age		Female		BMI		Hand Temperature		Weight		Height		Urine		Hair	
				β	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Log(Ulnar Peak Latency)	Base model [b]	-	0.36	2.12	0.002	*0.0001*	−0.049	*0.0001*	−0.001	*0.09*	−0.031	*0.0001*					0.006	*0.05*		
	GPX1(C>T) [a]	rs1050450	0.31	2.16	0.002	*0.0001*	−0.046	*0.0001*	−0.003	*0.02*	−0.031	*0.0002*					0.009	*0.07*		
Log(Ulnar Onset Latency)	Base model [b]	-	0.29	1.92	0.002	*0.0011*	−0.07	*0.0001*			−0.03	*0.0001*							−0.01	*0.08*
	MT1E 3′UTR(G>T) [a]	rs708274	0.31	2.13	0.002	*0.0006*	−0.07	*0.0001*			−0.04	*0.0001*							−0.019	*0.04*
Log(Sural Amplitude)	Base model [b]	-	0.25	4.92	−0.02	*0.0001*							−0.007	*0.0001*	−0.006	*0.03*			0.07	*0.06*
	GCLC 5′ UTR(G>A)	rs17883901	0.34	5.26	−0.01	*0.0001*							−0.008	*0.0003*	−0.008	*0.01*			0.03	*0.54*

			SNP main effects					SNP-Urine interactions				SNP-Hair interactions			
	SNP Name	db SNP	Major Homozygote	Heterozygote		Minor Homozygote		Urine X Heterozygote		Urine X Minor homozygote		Hair X Heterozygote		Hair X Minor homozygote	
			Ref	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Log(Ulnar Peak Latency)	Base model [b]	-	-	-	-	-	-	-	-	-	-				
	GPX1(C>T)	rs1050450	-	0.017	*0.23*	-	-	**−0.016**	***0.05***	-	-				
Log(Ulnar Onset Latency)	Base model [b]	-	-	-	-	-	-					-	-	-	-
	MT1E 3′UTR(G>T) [a]	rs708274	-	−0.048	*0.005*	-	-					**0.033**	***0.05***	-	-
Log(Sural Amplitude)	Base model [b]	-	-	-	-	-	-					-	-	-	-
	GCLC 5′ UTR(G>A)	rs17883901	-	−0.19	*0.09*	-	-					**0.32**	***0.01***	-	-

[a] Pooled heterozygotes and minor homozygotes were compared to major homozygotes

[b] Derived using both 2009 and 2010 data

Table 4

Mean Nerve Conduction Measurements Stratified by Genotypes of Selected SNPs

SNP name	db SNP	Mean Nerve Conduction Functions			
		Major homozygote (N)	Heterozygote(N)	Minor homozygote(N)	ANOVA *p*
		Ulnar Peak Latency (msec)			
GPX1(C>T)	rs1050450	3.26(107)	3.23(79)	3.18(21)	0.54
		Ulnar Onset Latency (msec)[a]			
MT1E 3′UTR(G>T)	rs708274	2.51(168)	2.46(48)	-	0.22
		Sural Amplitude (μV) [a]			
GCLC 5′ UTR(G>A)	rs17883901	13.23(183)	12.78(27)	-	0.74

Note:

[a]Due to small size in minor homozygote, the results shown are pooled results of minor homozygote with heterozygote.