Published in final edited form as:
J Toxicol Environ Health A. 2008 ; 71(19): 1318–1326. doi:10.1080/15287390802240850.

The Association Between Serotonin Transporter Gene Promoter Polymorphism (5-HTTLPR), Self-Reported Symptoms, and Dental Mercury Exposure

Nicholas J. Heyer[1], **Diana Echeverria**[1,2], **Federico M. Farin**[2], and **James S. Woods**[2]

1*Battelle Centers for Public Health Research and Evaluation, Seattle, Washington, USA*

2*Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington, USA*

Abstract

The associations between a polymorphism of the serotonin transporter gene (5-HTTLPR), dental mercury exposure, and self-reported symptoms were evaluated among 157 male dentists and 84 female dental assistants. Self-reported symptoms and detailed work histories were obtained by computerized questionnaire. Spot urine samples were collected and analyzed for mercury concentrations to evaluate recent exposures, whereas a chronic mercury exposure index was created from the work histories. 5-HTTLPR polymorphism status was determined using a polymerase chain reaction (PCR)-based assay. Scores for current, recent, and chronic self-reported symptom groups were evaluated with respect to recent and chronic mercury exposure and 5-HTTLPR polymorphism status. Multiple regression analysis controlled for age, socioeconomic status, tobacco and alcohol use, self-reported health problems, and medications. Analyses were restricted to Caucasian subjects due to the highly skewed distribution of the 5-HTTLPR polymorphism. Separate evaluations were conducted for dentists and dental assistants. In contrast to previous reports, no consistent associations were found between either urinary mercury concentration or the chronic index of mercury exposure and any category of symptoms. However, both significant and consistent associations were observed between increased symptoms and the 5-HTTLPR polymorphism involving two copies of the short or "s" allele (full mutation), but not with the polymorphism involving only one copy (heterozygous), demonstrating a gene–dose relationship for symptom reporting. These findings suggest that within this restricted population increased symptoms of depression, anxiety, and memory are associated with the 5-HTTLPR polymorphism among both males and females.

Historically, occupational exposures to high levels of elemental mercury (Hg^0) have been associated with potentially severe neurological deficits (Albers et al., 1988; Langworth et al., 1992; Pranjic et al., 2003; Piikivi et al., 1984; Piikivi & Hanninen, 1989), whereas more recent studies reported such effects at substantially lower levels of Hg^0 exposure (Clarkson et al., 2003; Langworth et al., 1997; Ngim et al., 1992; Ritchie et al., 2002; Shapiro et al., 1982; Soleo et al., 1990). Our own studies documented adverse neurobehavioral effects associated with elemental mercury exposures in the range of those experienced by the general population with mercury amalgam dental fillings (Bittner et al., 1998; Echeverria et al., 1995, 1998). At such low levels of exposure, it becomes prudent to control for individual factors that may influence the sensitivity to Hg^0-mediated effects. To this end, our previous studies described increased susceptibility to the adverse neurobehavioral effects of Hg^0 exposure among dentists

Address correspondence to Nicholas J. Heyer, PhD, Battelle Centers for Public Health Research and Evaluation, 1100 Dexter Ave. S., Suite 400, Seattle, WA 98109-3598, USA. E-mail: heyern@battelle.org.

and dental assistants expressing polymorphisms of genes encoding the heme biosynthetic pathway enzyme, coproporphyrinogen oxidase (CPOX) (Echeverria et al., 2006), and brain derived neurotrophic factor (BDNF) (Heyer et al., 2004; Echeverria et al., 2005). The polymorphism in exon 4 of the CPOX gene (CPOX4) exacerbates an abnormal urinary porphyrin excretion pattern associated with mercury exposure (Woods et al., 2005; Heyer et al., 2006), whereas the BDNF polymorphism has been linked with abnormal hippocampal activation and reduced performance on measures of memory (Egan et al., 2003; Hariri et al., 2003; Marx, 2003). Our studies showed independent and additive joint effects for these two polymorphisms and Hg^0 on a range of neurobehavioral functions in humans (Echeverria et al., 1995, 2005, 2006; Heyer et al., 2004).

Another variant of interest with respect to potentially modifying the neurobehavioral consequences of Hg^0 exposure in humans is a functional insertion/deletion polymorphism (5-HTTLPR) in the promoter region of the serotonin transporter (*SLC6A4*) gene. The 5-HTTLPR polymorphism is located on chromosome 17q11, and this region of the gene has the potential to regulate the level of the functional serotonin transporter (Lesch et al., 1996, 1999). Of the two primary alleles, a long or "l" allele has 16 repetitive sequences in the 5-HTTLPR, while the short or "s" allele has only 14 repetitions. In cells homozygous for the "l" allele (wild-type), serotonin uptake was found to be more than twofold higher compared to cells with one (heterozygous) or two (full mutant) copies of the short or "s" allele (Lesch et al., 1996). Studies implicated the "s" polymorphism with anxiety (Lesch et al., 1996; Murakami et al., 1999; Katsuragi et al., 1999; Schinka et al., 2004; Munafo et al., 2005), depressive symptoms (Osher et al., 2000; Hoefgen et al., 2005), neuroticism (Greenberg et al., 2000; Sen et al., 2004; Du et al., 2000), affective disorders (Hauser et al., 2003; Lasky-Su et al., 2005), and suicidal behavior (Li and He, 2007) in human subjects. The aim of this study was to examine the effects of the 5-HTTLPR polymorphism on the neurobehavioral consequences of low-level occupational Hg^0 exposure in the same dental professional population with specific attention to a standardized list of self-reported symptoms.

Materials and Methods

The Study Population

Dentists were identified from the registry of the Washington State Dental Association, and our initial contact included a short eligibility questionnaire and a request for a spot urine sample. Among the 1488 respondents who met our study criteria, mean urinary mercury concentrations (HgU) were 2.5 μg/L (range 0–67). A random stratified (based upon HgU concentrations) sample of 261 dentists (all male) was contacted, and 193 dentists (74%) with sufficient information for inclusion in our initial analyses were recruited into the present study. The methods of recruitment and eligibility criteria of the study population were previously described (Heyer et al., 2004; Woods et al., 2005).

Dental assistants (all females) were recruited independently from the practices of participating dentists to provide a similar range of dental office Hg^0 exposures. Four hundred potential dental assistant subjects were contacted, and 230 (58%) with sufficient information for inclusion in our initial analyses were recruited.

The current analyses are based on 163 dentists (85% of the initial cohort) and 101 dental assistants (44% of the original cohort) for whom it was possible to obtain 5-HTTLPR genotyping. The institutional review boards of Battelle and the University of Washington approved the study protocol. All subjects gave written consent prior to participating in the study.

Data Collection

Neurobehavioral tests were conducted at a central location, but some participants were tested at their offices due to distance from our test facility or their convenience. Participating subjects took a breath alcohol test prior to additional testing, and provided a ~50-ml spot urine sample for mercury analysis. Buccal cells were harvested from the inner cheek of each subject to provide DNA for genetic testing. Other test procedures included completing a computerized questionnaire (Neuroquest) and a computerized behavioral test battery, the Behavioral Evaluation for Epidemiologic Studies (BEES) (Echeverria et al., 2002). In addition, various paper-and-pencil and stand-alone neurological tests were also administered. Evaluations of behavioral performance and mood scales will be reported in a companion article.

Neuroquest collects information on demographics and personal habits, use of vitamins and supplements, medical and pregnancy histories, work histories, a symptoms checklist (45 symptoms), and a computerized version of the Profile Of Mood States (McNair et al., 1971) to measure current mood. Medical conditions are grouped into categories including (1) physical injury, (2) major operations, (3) digestive problems, (4) circulation problems, (5) sensory problems, (6) kidney problems, (7) endocrine problems, (8) immune problems, (9) brain-related problems, and (10) emotional problems. Yes/no information was collected on the presence of any condition, the presence of specific or "other" conditions, and the current use of medications for any of these conditions within each category.

Urinary Mercury Analyses

Analysis of total mercury in urine samples was performed using continuous-flow, cold-vapor spectrofluorometry, as previously described (Pingree et al., 2001). Urine samples were analyzed in triplicate and the geometric mean of the three analyses was computed as the Hg concentration of the sample. Urinary Hg levels were calculated as micrograms per liter of urine.

5-HTTLPR Genotyping

Genotyping was performed by the Functional Genomics Laboratory of the Center for Ecogenetics and Environmental Health at the University of Washington. The procedure employed a polymerase chain reaction (PCR)-based assay to identify the "long" and "short" polymorphic alleles that are located in the promoter region of the 5-HTT gene, which consists of the insertion/deletion of 44 base pairs. The PCR primers were as follows: sense strand: 5′-ggCgTTgCCgCTCTgAATgC-3′ and antisense strand: 5′-gAgggACTgAgCTggACAACCAC-3′. The PCR products were resolved on an ethidium bromide-stained 3% agarose gel, sized using DNA molecular weight markers, visualized with ultraviolet (UV) light, and the alleles were identified as previously described (Woods et al., 2005; Heyer et al., 2004). 5-HTTLPR alleles were classified as long or "l" type if there were 16 sequence repetitions and as short or "s" type if there were only 14 repetitions. Samples were considered wild-type (in their natural form) if they had two "l" alleles (ll), heterozygous if they had one "l" and one "s" allele (ls), and "full mutation" if they contained two "s" alleles (ss).

Mercury Exposure Scores

Recent mercury exposure was calculated as the natural log of the spot concentration of mercury in urine measures as micrograms per liter. The log is generally considered to have a better linear association with toxic effects. Before conversion, the value 1 was added to each concentration so that a nondetectable urinary concentration of mercury would have the value of its natural log be zero, thus avoiding the analyses being overwhelmed by large differences in the value of the log for very small differences in urinary concentrations of less than 1 μg/L.

The chronic measure of mercury exposure is not based on the measured urinary levels, in part, to keep them from being too highly correlated. Historical mercury exposure for each self-reported job was calculated using the product of the reported average number of mercury amalgam fillings or removals performed weekly and the duration of the job. This product was then weighted by a time-period factor [1 ≥ 1992, 1.5 = 1985–1992, 1.75 ≥ 1972–1982, 2.0 ≤ 1970] based upon historically measured urinary mercury levels in dental populations. Each subject's chronic mercury exposure index was created by taking the square root of the sum the contributions of each of their self-reported dental jobs.

Symptoms Scores

Self-reported symptoms were obtained through a computerized symptom checklist of our design that is described elsewhere (Heyer et al., 2004). This checklist distinguishes between current (today) and recent (over the past 3 mo) symptoms. Recent symptoms are further classified by their duration, allowing the definition of chronic symptoms. A total of 45 symptoms are included, but only 27 are used to define the current checklist, as they evaluate activities over time (e.g., “write notes to remind myself”). Current symptoms were scored for intensity (range 0–4), while recent symptoms were scored using the product of their intensity and frequency (0–20). Chronic symptoms were defined as those recent symptoms lasting at least 1 yr. Thus, chronic symptoms are statistically tied to recent symptoms, and should not be considered totally independent.

In order to reduce the number of symptom variables in these analyses, 12 a priori symptom groups were created, each assigned a score equal to the highest (maximum) individual symptom score within that group. This “maximum” score was used to avoid very high scores associated with subjects who ‘globalized’ their symptom reporting. In addition, three individual symptom questions were selected to use independently of their group because they seemed to capture the full concept of the symptom group (these included memory, confusion and depression). These are reported as their group name with “1 variable” attached. The 12 symptom groups are: memory, confusion, depression, anxiety, coordination, mood, headache, parasthesias, muscle symptoms, stomach symptoms, skin symptoms, and lung symptoms. Thus, in total 15 symptom outcomes, which are not necessarily statistically independent, were evaluated.

Analyses

Analyses were conducted on a restricted group of dentists and dental assistants because of an unbalanced distribution of the 5-HTTLPR polymorphism. Table 1 shows that among non-Caucasian members of our study population, there were no dentists designated as 5-HTTLPR wild-type (ll), and only one such designee was identified among dental assistants. Thus, non-Caucasian members of this cohort, including 6 males and 17 females, were excluded from the final analyses. Furthermore, among Caucasian members, there was a distinct difference in the percentages designated as wild-type (ll) and heterozygous (ls) between males and females. This difference, along with the differences in Hg^0 exposure levels, age structures, and symptom reporting profiles, prompted us to conduct separate analyses for males and females, as presented in Table 2. Notably, racial/ethnic differences in the distribution of the 5-HTTLPR polymorphism were previously reported (Kunugi et al., 1997;Ishiguro et al., 1997).

Cross-sectional analyses were conducted using SPSS (version 15.0 for Windows). A data file was constructed that contained all symptom scores, measures of exposure to elemental mercury (both current and chronic), the 5-HTTLPR genotyping results, and covariates. Potential covariates evaluated in the analyses included demographic, dietary, and medical history variables.

Final regression analyses included evaluation of both base and full models. The base model is comprised of age, ln urinary mercury concentration (HgU), the chronic mercury exposure index, and dummy variables for either the heterozygous (ls) and full mutation (ss) polymorphism of 5-HTTLPR. Subjects with wild-type (ll) polymorphisms had both dummy variables set to zero. The full model included all the variables listed in Table 2 and employed backward deletion using a conservative elimination *p* value of .2 (Budtz-Jorgensen et al., 2007). Results are reported for both the "base" and "full" models, because our cross-sectional data made the causal inference for observed associations ambiguous, and it was apparent that self-reported medical histories could indicate either a causal factor for a symptom or a result of having that symptom. These measures therefore conferred an element of caution towards the potential for over-controlling in the analyses.

Results

The final cohort for this analysis comprised 157 Caucasian male dentists (DTs) and 84 Caucasian female dental assistants (DAs). This number of subjects is smaller than previously reported (Heyer et al., 2004), reflecting primarily our ability to obtain confirmed 5-HTTLPR genotyping results for only 85% of the dentists and 44% of the dental assistants involved in that study, and our elimination of 23 non-Caucasian subjects (as discussed earlier; see Table 1). The smaller number of subjects most likely reduced our ability to observe statistically significant changes associated with exposure within the large personal variability found in most human populations. Comparing differences between our previously reported cohort (Heyer et al., 2004) and our current study cohort (by gender) shows no statistically significant differences in the means for age, vocabulary, income or urinary mercury levels. However, in the current study, mean urinary mercury levels were quantitatively higher for male dentists (+0.09 μg/L), and lower for female dental assistants (−0.22 μg/L).

Table 2 shows the distributions among Caucasian subjects for exposures and covariates employed in this study. Dentists were significantly older than dental assistants, and had greater vocabularies (a measure of training and education). Dentists also drank and smoked more than dental assistants, although these measures showed only moderate use of these substances. In terms of medical histories, dentists were far more likely to have a history of cancer, and dental assistants were more likely to have immunological problems and to take allergy medications. While the correlation of average urinary mercury (HgU) concentrations between dentists and dental assistants from the same office was significant, dentists had significantly higher exposures to mercury as measured by both HgU and our chronic exposure index. These clear occupation/gender differences support our decision to analyze the two populations separately, rather than attempting to control for occupation/gender in the analysis.

Table 3 shows the distributions of the symptom category scores among dentists (DTs) and dental assistants (DAs). Symptoms are reported both as the percent reporting any symptom and the average symptom score for each category. Dental assistants generally reported higher levels of symptoms than did dentists. In particular, dentists reported low symptom levels related to coordination and muscle (which are directly related to their jobs). High levels of anxiety and depression were reported for both dentists and dental assistants.

Table 4 shows the outcomes of regression analyses modeling symptom scores for these categories. Because of the many possible outcomes, and to make the table easier to read, only those results with significance for either the base or full model at $p < .05$ are shown (other results are not listed or are shaded out). Interestingly, by comparing results from the base and full models, it is observed that the covariates evaluated had relatively limited impact on the associations between the recent or chronic indices of Hg^0 exposure or 5-HTTLPR polymorphism status and symptoms. In some cases the covariates tended to dilute significance,

and in a few cases they increased significance, but the overall effect remains fairly stable across models.

Recent Mercury Exposure and Symptoms

Among dentists (DTs), recent mercury exposure, as measured by urinary mercury concentration, was significantly associated with six symptom categories: three for symptoms experienced "today," one for "recent" symptoms, and two for "chronic" symptoms (Table 4). However, all but one of these associations showed symptoms decreasing with increasing Hg^0 exposure. The one positive association between symptoms and exposure was with the symptom category "anxiety today," which was significant only when the covariates were introduced.

Among dental assistants (DAs), there were again six symptom categories that were associated with recent mercury exposure. Four of these associations were positive, including two measures for "confusion today," one for "recent headache," and one for "chronic headache" (these latter two are closely correlated). The two negative associations included measures of "chronic confusion." There was little concordance with any of the findings among dentists. The associations for "confusion today" in dental assistants were matched to significant finding in the opposite direction for dentists, while the associations with "chronic confusion" were negative in both groups.

Chronic Mercury Exposure and Symptoms

As shown in Table 4, there were only two observed significant associations between the chronic mercury index and symptoms, both among dentists. One symptom, "anxiety today," was positive, while the other, "chronic skin symptoms," was negative.

Heterozygous Form of the 5-HTTLPR Polymorphism (ls) and Symptoms

The two significant associations between the heterozygous form of 5-HTTLPR polymorphism and symptoms were both negative. These were for "anxiety today" and "lung symptoms today" among dentists.

Full Mutation Form of the 5-HTTLPR Polymorphism (ss) and Symptoms

Among dentists, there were seven observed associations between the full mutation form of 5-HTTLPR polymorphism (ss) and symptoms, all positive. Five were with "recent" symptoms, and two were with "chronic" symptoms. The "recent" symptoms included two measures of depression, anxiety, memory, and moodiness. One of the depression measures was near significance in the base model and reached significance in the full model, while the anxiety measure was significant in the base model, but lost significance in the full model. The other three were significant in both models. The "chronic" symptoms included memory and headache, with headache reaching significance only in the full model.

Among dental assistants, there were nine observed associations between the mutation form of 5-HTTLPR polymorphism and symptoms, all positive. Four were with "recent" symptoms and five were with "chronic" symptoms. The "recent" symptoms included measures of depression, anxiety, memory and skin symptoms, with memory only reaching significance in the base model. The "chronic" symptoms included two measures of depression, anxiety, memory, and skin, with anxiety and memory only reaching significance in the base model.

There was considerable concordance between dentists and dental assistants, with both occupational/gender groups having significant associations with symptoms of depression, anxiety, and memory in both the "recent" and "chronic" symptom categories. The dental

assistants also had significant associations with symptoms of the skin for both the "recent" and "chronic" symptom categories.

Interactions Between Mercury Exposure and 5-HTTPLR Polymorphism

No significant interactions between mercury exposure and the 5-HTTPLR polymorphism were found for any category of symptom evaluated. Notably, within the "today" category, "anxiety" was positively associated with the chronic mercury index, but negatively associated with the heterozygous form of the 5-HTTLPR polymorphism. Not surprisingly, evaluation of multiplicative interaction terms showed no significance.

Discussion

This study found no consistent associations between indices of Hg^0 exposure and self-reported symptoms among male or female dental professionals with prolonged low-level occupational exposure to dental amalgam mercury. These findings are in contrast to those previously reported (Heyer et al., 2004) wherein numerous associations were observed between both recent and chronic Hg^0 exposure indices and all categories of symptoms among both dentists (males) and dental assistants (females), principally among the latter. While the present findings replicate previously observed associations between recent mercury exposure and confusion today among dental assistants and between the chronic index of mercury exposure and anxiety today among dentists from our previous analyses, they do not replicate associations between chronic exposure index and "recent" symptoms among dental assistants.

These results demonstrate the difficulties in reproducing behavioral or symptomatic results for very low exposures among different populations and with changing explanatory variables. The inclusion of the 5-HTTLPR polymorphism in the analyses may have affected the current results, although its correlations with exposure were low. The correlations with the homozygous mutant (ss) polymorphism were −.069 (p = .392) for dentists and .131 (p = .239) for dental assistants. The correlations with the heterozygous (ls) polymorphism were somewhat higher at −.106 (p = .185) and −.197 (p = .075), respectively. However, the more likely explanation for this outcome is the smaller size of the current study cohort, which reduced our ability to explain the natural variance within the population. Another consideration is the exclusion of non-Caucasian members of the overall cohort from the present analysis, inclusion of which may have influenced the previous results.

Notably, however, consistent and significant associations were observed between increased symptoms and the 5-HTTLPR polymorphism involving two copies of the short or "s" allele (full mutation, ss), but not with that involving only one copy (heterozygous, ls). These findings are consistent with others also reporting significant differences between the double "s" allele vs. the combined heterozygous and wildtype variants of the 5-HTTLPR polymorphism and with effects prominently observed among women (Melke et al., 2001; Munafo et al., 2005). Symptoms associated with the full mutant 5-HTTLPR polymorphism in the present study, especially depression and anxiety, are consistent with the types of associations reported in the literature. Previous studies linked the presence of this polymorphism with anxiety, depression, neuroticism, affective disorders, and suicidal behavior (Lesch et al., 1996; Greenberg et al., 2000; Hoefgen et al., 2005; Du et al., 2000). While there have also been many negative studies, several meta-analyses have supported these findings (Schinka et al., 2004; Munafo et al., 2005).

The present findings are of interest in terms of further defining individual sensitivity to Hg^0 neurotoxicity in humans as well as in identifying future research needs. The present results add to evidence from previous studies (Echeverria et al., 2005, 2006) demonstrating increased sensitivity to Hg^0 associated with other genetic polymorphisms that selectively impair

neurobehavioral functions that are known to be concomitantly affected by Hg^0 exposure. While the present findings do not demonstrate additive effects of the 5-HTTLPR polymorphism and Hg^0 on the specific outcome measures evaluated here, it is reasonable to assume that such effects would be observed in relation to broader measures of mood and behavior, as previously reported. Further studies of the effects of the 5-HTTLPR polymorphism in association with Hg^0 exposure on a wide spectrum of neurobehavioral parameters, as evaluated by the BEES test battery (Echeverria et al., 2002), within the present occupationally exposed dental population are in progress. Of additional importance is the assessment the effects of the 5-HTTLPR and other polymorphisms on sensitivity to Hg^0 toxicity in children, perhaps the most susceptible segment of the human population. Notably, evidence for a role of 5-HTT in the integration of synaptic connections in the mammalian brain during development (Lesch & Mossner, 1998) suggests that children with the 5-HTTLPR polymorphism and concomitant Hg^0 exposure may be at particular risk. Ongoing studies will evaluate this possibility by performing genotyping assays for the 5-HTTLPR polymorphism on DNA acquired from children and determining the potential effects of 5-HTTLPR on Hg-mediated neurological and neurobehavioral effects.

In conclusion, the Neuroquest computerized self-reported symptom severity checklist employed in this study was shown to be sensitive to 5-HTTLPR polymorphism status and disclosed consistent associations between the mutant form of this polymorphism and symptoms of depression, anxiety, and memory for both male and female subjects. This finding is consistent with those of other studies both with respect to the type of symptoms and with the homozygous mutant form (with two copies of the “s” allele) driving the associations. In contrast to previous findings, no consistent associations were observed between either recent or chronic indices of Hg^0 exposure in the present study, nor were any significant interactions between 5-HTTPLR status and Hg^0 exposure observed. However, this study demonstrates that this common polymorphism influences the reporting of symptoms and should be considered when conducting studies using symptoms as outcomes.

Acknowledgements

This research was supported by Center Grant P30ES07033 and by Superfund Program Project Grant P42ES04696 to the University of Washington from the National Institute of Environmental Health Sciences (NIEHS), National Institues of Health. Additional funding was provided by the Wallace Research Foundation.

References

Albers JW, Kallenbach LR, Fine LJ, Langolf GD, Wolfe RA, Donofrio PD, Alessi AG, Stolp-Smith KA, Bromberg MB. Neurological abnormalities associated with remote occupational elemental mercury exposure. Ann Neurol 1988;24:651–659. [PubMed: 2849369]

Bittner AC Jr, Echeverria D, Woods JS, Aposhian HV, Naleway C, Martin MD, Mahurin RK, Heyer NJ, Cianciola M. Behavioral effects of low-level exposure to Hg^0 among dental professionals: A cross-study evaluation of psychomotor effects. Neurotoxicol Teratol 1998;20:429–439. [PubMed: 9697969]

Budtz-Jorgensen E, Keiding N, Grandjean P, Weihe P. Confounder selection in environmental epidemiology: Assessment of health effects of prenatal mercury exposure. Ann Epidemiol 2007;17:27–35. [PubMed: 17027287]

Clarkson TW, Magos L, Myers GJ. The toxicology of mercury—Current exposures and clinical manifestations. N Engl J Med 2003;349:1731–1737. [PubMed: 14585942]

Du L, Bakish D, Hrdina PD. Gender differences in association between serotonin transporter gene polymorphism and personality traits. Psychiat Genet 2000;10:159–164.

Echeverria D, Aposhian HV, Woods JS, Heyer NJ, Aposhian MM, Bittner AC Jr, Mahurin RK, Cianciola M. Neurobehavioral effects from exposure to dental amalgam Hg^0: New distinctions between recent exposure and Hg body burden. FASEB J 1998;12:971–980. [PubMed: 9707169]

Echeverria D, Heyer NJ, Bittner AC Jr, Rohlman D, Woods JS. Test-retest reliability and factor stability of the behavioral evaluation for epidemiology studies test battery. Percept Motor Skills 2002;95:845–867. [PubMed: 12509185]

Echeverria D, Heyer NJ, Martin MD, Naleway CA, Woods JS, Bittner AC Jr. Behavioral effects of low-level exposure to elemental Hg among dentists. Neurotoxicol Teratol 1995;17:161–168. [PubMed: 7760775]

Echeverria D, Woods JS, Heyer NJ, Rohlman D, Farin FM, Li T, Garabedian CE. The association between a genetic polymorphism of coproporphyrinogen oxidase, dental mercury exposure and neurobehavioral response in humans. Neurotoxicol Teratol 2006;28:39–48. [PubMed: 16343843]

Echeverria D, Woods JS, Heyer NJ, Rohlman DS, Farin FM, Bittner AC Jr, Li T, Garabedian C. Chronic low-level mercury exposure, BDNF polymorphism, and associations with cognitive and motor function. Neurotoxicol Teratol 2005;27:781–796. [PubMed: 16301096]

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 2003;112:257–269. [PubMed: 12553913]

Greenberg BD, Li Q, Lucas FR, Hu S, Sirota LA, Benjamin J, Lesch KP, Hamer D, Murphy DL. Association between the serotonin transporter promoter polymorphism and personality traits in a primarily female population sample. Am J Med Genet 2000;96:202–216. [PubMed: 10893498]

Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF, Weinberger DR. Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. J Neurosci 2003;23:6690–6694. [PubMed: 12890761]

Hauser J, Leszczynska A, Samochowiec J, Czerski PM, Ostapowicz A, Chlopocka M, Horodnicki J, Rybakowski JK. Association analysis of the insertion/deletion polymorphism in serotonin transporter gene in patients with affective disorder. Eur Psychiat 2003;18:129–132.

Heyer NJ, Bittner AC Jr, Echeverria D, Woods JS. A cascade analysis of the interaction of mercury and coproporphyrinogen oxidase (CPOX) polymorphism on the heme biosynthetic pathway and porphyrin production. Toxicol Lett 2006;161:159–166. [PubMed: 16214298]

Heyer NJ, Echeverria D, Bittner AC Jr, Farin FM, Garabedian CC, Woods JS. Chronic low-level mercury exposure, BDNF polymorphism, and associations with self-reported symptoms and mood. Toxicol Sci 2004;81:354–363. [PubMed: 15254338]

Hoefgen B, Schulze TG, Ohlraun S, von Widdern O, Hofels S, Gross M, Heidmann V, Kovalenko S, Eckermann A, Kolsch H, Metten M, Zobel A, Becker T, Nothen MM, Propping P, Heun R, Maier W, Rietschel M. The power of sample size and homogenous sampling: association between the 5-HTTLPR serotonin transporter polymorphism and major depressive disorder. Biol Psychiat 2005;57:247–251. [PubMed: 15691525]

Ishiguro H, Arinami T, Yamada K, Otsuka Y, Toru M, Shibuya H. An association study between a transcriptional polymorphism in the serotonin transporter gene and panic disorder in a Japanese population. Psychiat Clin Neurosci 1997;51:333–335.

Katsuragi S, Kunugi H, Sano A, Tsutsumi T, Isogawa K, Nanko S, Akiyoshi J. Association between serotonin transporter gene polymorphism and anxiety-related traits. Biol Psychiat 1999;45:368–370. [PubMed: 10023516]

Kunugi H, Hattori M, Kato T, Tatsumi M, Sakai T, Sasaki T, Hirose T, Nanko S. Serotonin transporter gene polymorphisms: Ethnic difference and possible association with bipolar affective disorder. Mol Psychiat 1997;2:457–462.

Langworth S, Almkvist O, Soderman E, Wikstrom BO. Effects of occupational exposure to mercury vapour on the central nervous system. Br J Ind Med 1992;49:545–555. [PubMed: 1515346]

Langworth S, Sallsten G, Barregard L, Cynkier I, Lind ML, Soderman E. Exposure to mercury vapor and impact on health in the dental profession in Sweden. J Dent Res 1997;76:1397–1404. [PubMed: 9207773]

Lasky-Su JA, Faraone SV, Glatt SJ, Tsuang MT. Meta-analysis of the association between two polymorphisms in the serotonin transporter gene and affective disorders. Am J Med Genet B Neuropsychiat Genet 2005;133:110–115.

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996;274:1527–1531. [PubMed: 8929413]

Lesch KP, Jatzke S, Meyer J, Stober G, Okladnova O, Mossner R, Riederer P. Mosaicism for a serotonin transporter gene promoter-associated deletion: Decreased recombination in depression. J Neural Transm 1999;106:1223–1230. [PubMed: 10651116]

Lesch KP, Mossner R. Genetically driven variation in serotonin uptake: Is there a link to affective spectrum, neurodevelopmental and neurodegenerative disorders? Biol Psychiat 1998;44:179–192. [PubMed: 9693390]

Li D, He L. Meta-analysis supports association between serotonin transporter (5-HTT) and suicidal behavior. Mol Psychiat 2007;12:47–54.

Marx J. Neuroscience. Minor variation in growth-factor gene impairs human memory. Science 2003;299:639–640. [PubMed: 12560519]

McNair, DM.; Lorr, M.; Droppleman, LF. Profile of mood states. San Diego: Educational and Industrial Testing Service; 1971.

Melke J, Landen M, Baghei F, Rosmond R, Holm G, Bjorntorp P, Westberg L, Hellstrand M, Eriksson E. Serotonin transporter gene polymorphisms are associated with anxiety-related personality traits in women. Am J Med Genet 2001;105:458–463. [PubMed: 11449399]

Munafo MR, Clark T, Flint J. Does measurement instrument moderate the association between the serotonin transporter gene and anxiety-related personality traits? A meta-analysis Mol Psychiat 2005;10:415–419.

Murakami F, Shimomura T, Kotani K, Ikawa S, Nanba E, Adachi K. Anxiety traits associated with a polymorphism in the serotonin transporter gene regulatory region in the Japanese. J Hum Genet 1999;44:15–17. [PubMed: 9929970]

Ngim CH, Foo SC, Boey KW, Jeyaratnam J. Chronic neurobehavioural effects of elemental mercury in dentists. Br J Ind Med 1992;49:782–790. [PubMed: 1463679]

Osher Y, Hamer D, Benjamin J. Association and linkage of anxiety-related traits with a functional polymorphism of the serotonin transporter gene regulatory region in Israeli sibling pairs. Mol Psychiat 2000;5:216–219.

Piikivi L, Hanninen H. Subjective symptoms and psychological performance of chlorine-alkali workers. Scand J Work Environ Health 1989;15:69–74. [PubMed: 2922591]

Piikivi L, Hanninen H, Martelin T, Mantere P. Psychological performance and long-term exposure to mercury vapors. Scand J Work Environ Health 1984;10:35–41. [PubMed: 6740275]

Pingree SD, Simmonds PL, Woods JS. Effects of 2,3-dimercapto-1-propanesulfonic acid (DMPS) on tissue and urine mercury levels following prolonged methylmercury expsoure in rats. Toxicol Sci 2001;61:224–233. [PubMed: 11353131]

Pranjic N, Sinanovic O, Jakubovic R. Chronic psychological effects of exposure to mercury vapour among chlorine-alkali plant workers. Med Lav 2003;94:531–541. [PubMed: 14768245]

Ritchie KA, Gilmour WH, Macdonald EB, Burke FJ, McGowan DA, Dale IM, Hammersley R, Hamilton RM, Binnie V, Collington D. Health and neuropsychological functioning of dentists exposed to mercury. Occup Environ Med 2002;59:287–293. [PubMed: 11983843]

Schinka JA, Busch RM, Robichaux-Keene N. A meta-analysis of the association between the serotonin transporter gene polymorphism (5-HTTLPR) and trait anxiety. Mol Psychiat 2004;9:197–202.

Sen S, Burmeister M, Ghosh D. Meta-analysis of the association between a serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits. Am J Med Genet B Neuropsychiat Genet 2004;127:85–89.

Shapiro IM, Cornblath DR, Sumner AJ, Uzzell B, Spitz LK, Ship II, Bloch P. Neurophysiological and neuropsychological function in mercury-exposed dentists. Lancet 1982;1:1147–1150. [PubMed: 6122938]

Soleo L, Urbano ML, Petrera V, Ambrosi L. Effects of low exposure to inorganic mercury on psychological performance. Br J Ind Med 1990;47:105–109. [PubMed: 2310714]

Woods JS, Echeverria D, Heyer NJ, Simmonds PL, Wilkerson J, Farin FM. The association between genetic polymorphisms of coproporphyrinogen oxidase and an atypical porphyrinogenic response to mercury exposure in humans. Toxicol Appl Pharmacol 2005;206:113–120. [PubMed: 15967199]

Table 1

5-HTTLPR Genotype by Gender and Race

Gender	Genotype	Caucasian	Non-Caucasian
Male	Wild-type (ll)	66 (42.0%)[a]	0 (0%)
	Heterogeneous (ls)	62 (39.5%)[a]	3 (50%)
	Mutant (ss)	29 (18.5%)	3 (50%)
	Total	157	6
Female	Wild-type (ll)	20 (23.8%)[a]	1 (5.9%)
	Heterogeneous (ls)	48 (57.1%)[a]	8 (47.1%)
	Mutant (ss)	16 (19.0%)	8 (47.1%)
	Total	84	17

[a]Gender differences significant at $p < .05$.

Table 2

Distributions of Covariates for Male Dentists (DTs) and Female Dental Assistants (DAs)

Continuous variables	DTs (*N* = 157) Range	DTs (*N* = 157) Mean	DTs (*N* = 157) SD	DAs (*N* = 84) Range	DAs (*N* = 84) Mean	DAs (*N* = 84) SD
Exposures						
Urinary mercury (μg/L)	0–15.6	2.5[a]	2.1	0–9.8	1.6[a]	1.7
Chronic Mercury Index	0–116.8	28.1[a]	21.1	0–50.8	15.5[a]	11.2
Covariates						
Age at evaluation	28–65	48.9[a]	7.7	20–56	36.5[a]	8.5
Vocabulary	7–11	10.6[a]	0.9	4–11	8.2[a]	2.0
Alcohol drinks/week	0–20	3.8[a]	4.0	0–20	2.4[a]	2.9
Cigarettes: *n*/day	0–10	0.11[a]	0.8	0–10	0.7[a]	2.2
Cigarettes: pack-years	0–25	1.8	4.5	0–30	1.6	4.2

Dichotomous covariates	DTs (*N* = 157) *n*	DTs (*N* = 157) %	DAs (*N* = 84) *n*	DAs (*N* = 84) %
Self-reported				
Alcohol problem—ever told	6	3.8	1	1.2
Physical impairment	9	5.7	7	8.3
Take allergy medications	27	17.2[a]	30	35.7[a]
Medical history of:				
Cancer	14	8.9[a]	2	2.4[a]
Physical problems	97	61.8	45	53.6
Emotional problems	9	5.7	8	9.5
Brain problems	9	5.7	6	7.1
Sensory problems	19	12.1	12	14.3
Circulatory problems	26	16.6	11	13.1
Kidney problems	7	4.5	4	4.8
Endocrine problems	8	5.1	8	9.5
Respiratory problems	20	12.7	14	16.7
Digestive problems	27	17.2	17	20.2
Immune system problems	5	3.2[a]	11	13.1[a]

[a] Gender differences $p < .05$.

Table 3

Distribution of Symptom Outcomes

Symptom group	Male DTs (*N* = 157)				Female DAs (*N* = 84)			
	Range	*n* (%) > 0	Mean	SD	Range	*n* (%) > 0	Mean	SD
Symptoms today (0–4)								
Confusion one variable	0–2	17 (11)	0.11	0.34	0–3	25 (30)	0.36	0.63
Depression one variable	0–2	16 (10)	0.11	0.33	0–2	10 (12)	0.14	0.42
Confusion	0–2	18 (12)	0.12	0.35	0–3	27 (32)	0.43	0.73
Depression	0–3	52 (33)	0.43	0.67	0–2	50 (60)	0.73	0.68
Anxiety	0–3	51 (33)	0.38	0.62	0–4	37 (44)	0.69	0.97
Coordination	0–2	3 (2)	0.03	0.19	0–4	14 (17)	0.25	0.67
Moody	0–3	9 (6)	0.08	0.35	0–1	15 (18)	0.18	0.39
Headache	0–2	19 (12)	0.13	0.38	0–4	19 (23)	0.35	0.75
Parasthesias	0–2	18 (12)	0.12	0.35	0–3	19 (23)	0.32	0.70
Muscle	0–3	4 (3)	0.04	0.28	0–1	12 (14)	0.14	0.35
Stomach	0–2	21 (13)	0.15	0.39	0–3	27 (32)	0.43	0.70
Skin	0–3	32 (20)	0.25	0.55	0–4	51 (61)	0.94	0.96
Lung	0–3	19 (12)	0.15	0.47	0–3	32 (38)	0.49	0.72
Recent symptoms (0–20)								
Memory one variable	0–12	68 (43)	1.06	1.79	0–16	54 (64)	1.90	2.76
Confusion one variable	0–6	37 (24)	0.43	0.93	0–20	39 (46)	1.58	3.00
Depression one variable	0–9	63 (40)	0.78	1.32	0–16	40 (48)	1.90	2.87
Memory	0–20	109 (69)	2.80	3.45	0–20	74 (88)	4.01	4.25
Confusion	0–12	44 (28)	0.62	1.41	0–20	46 (55)	1.95	3.26
Depression	0–16	122 (78)	2.84	3.10	0–20	76 (91)	5.94	4.92
Anxiety	0–12	119 (76)	2.51	2.61	0–20	77 (92)	5.52	5.11
Coordination	0–12	11 (7)	0.20	1.13	0–20	39 (46)	2.12	4.12
Moody	0–9	56 (36)	0.89	1.64	0–16	58 (69)	2.58	3.08
Headache	0–12	96 (61)	1.22	1.61	0–16	63 (75)	3.02	3.42
Parasthesias	0–12	41 (26)	0.68	1.63	0–15	29 (35)	1.63	3.41
Muscle	0–12	14 (9)	0.25	1.18	0–9	22 (26)	0.98	2.12
Stomach	0–6	86 (55)	1.20	1.55	0–16	67 (80)	3.30	3.28
Skin	0–9	51 (33)	0.86	1.67	0–20	56 (67)	3.95	4.92
Lung	0–9	48 (31)	0.66	1.34	0–12	47 (56)	1.94	2.85
Chronic symptoms (0–20)								
Memory one variable	0–12	66 (42)	1.05	1.79	0–12	42 (50)	1.42	2.31
Confusion one variable	0–6	34 (22)	0.41	0.93	0–20	31 (37)	1.36	3.01
Depression one variable	0–9	52 (33)	0.66	1.27	0–16	30 (36)	1.56	2.83
Memory	0–20	102 (65)	2.75	3.48	0–20	63 (75)	3.68	4.29
Confusion	0–6	36 (23)	0.49	1.06	0–20	38 (45)	1.71	3.26
Depression	0–16	109 (69)	2.66	3.11	0–20	66 (79)	5.29	5.12
Anxiety	0–12	96 (61)	1.83	2.34	0–20	65 (77)	4.55	4.90
Coordination	0–6	8 (5)	0.10	0.57	0–16	25 (30)	1.20	3.10
Mood	0–8	44 (28)	0.67	1.38	0–16	51 (61)	2.38	3.15
Headache	0–12	80 (51)	1.04	1.60	0–16	52 (62)	2.69	3.54
Parasthesias	0–12	33 (21)	0.59	1.60	0–15	23 (27)	1.21	2.85
Muscle	0–12	8 (5)	0.18	1.12	0–8	16 (19)	0.70	1.83
Stomach	0–6	72 (46)	1.06	1.55	0–16	62 (73)	3.13	3.35
Skin	0–9	43 (27)	0.76	1.65	0–20	49 (58)	3.60	4.97
Lung	0–6	33 (21)	0.40	0.98	0–12	39 (46)	1.77	2.90

Note. Current symptoms are scored for intensity (range 0–4), while recent symptoms are scored using the product of their intensity and frequency (0–20). Chronic symptoms are defined as those recent symptoms lasting at least 1 yr.

J Toxicol Environ Health A. Author manuscript; available in PMC 2008 December 30.

Table 4

Self-Reported Symptoms, Mercury Exposure, and 5-HTTLPR Polymorphism

		DTs (*N*=157)				DAs (*N*=84)			
		Base model		Full model		Base model		Full model	
Symptom	**Exposure**	**Beta**	**Sig.**	**Beta**	**Sig.**	**Beta**	**Sig.**	**Beta**	**Sig.**
Today									
Confusion 1 variable	Recent mercury	(−.163)	(**.047**)	(−.188)	(**.019**)	*.166*	*.156*	.247	**.031**
Confusion		(−.168)	(**.041**)	(−.171)	(**.032**)	*.151*	*.195*	.255	**.015**
Skin		*.096*	*.243*	.145	**.042**				
Anxiety	Chronic mercury	.234	**.013**	.220	**.019**				
Anxiety	5-HTTLPR heterogeneous	(−.202)	(**.022**)	(−.224)	(**.012**)				
Lung		*(−.148)*	*(.098)*	(−.170)	(**.049**)				
Recent									
Confusion 1 variable	Recent mercury	(−.170)	(**.038**)	(−.181)	(**.022**)				
Headache						.256	**.026**	.278	**.005**
Depression 1 variable	5-HTTLPR full mutation	.193	**.028**	.235	**.005**				
Depression		*.173*	*.057*	.182	**.042**	.456	**.001**	.428	**.001**
Anxiety		.197	**.030**	*.147*	*.094*	.326	**.015**	.264	**.025**
Memory		.180	**.048**	.179	**.048**	.307	**.025**	*.241*	*.068*
Moody		.229	**.011**	.217	**.015**				
Skin						.308	**.022**	.233	**.044**
Chronic									
Confusion 1 variable	Recent mercury	*(−.150)*	*(.068)*	(−.161)	(**.042**)	(−.235)	(**.045**)	*(−.186)*	*(.092)*
Confusion						(−.231)	(**.049**)	*(−.199)*	*(.062)*
Headache						.278	**.015**	.236	**.011**
Stomach		(−.167)	(**.039**)	(−.176)	(**.026**)				
Skin	Chronic mercury	*(−.125)*	*(.195)*	(−.161)	(**.048**)				
Depression 1 variable	5-HTTLPR full mutation					.273	**.045**	.296	**.023**
Depression						.465	**.000**	.420	**.001**
Anxiety						.314	**.021**	*.231*	*.052*
Memory		.186	**.041**	.196	**.030**	.314	**.021**	*.250*	*.067*
Headache		*.168*	*.062*	.206	**.018**				
Skin						.364	**.007**	.259	**.032**

Note. Only gender-specific categories with significant associations in either the “Base” or “Full” models are shown.

Boldface numbers indicate significant ($p < .05$); italicized numbers indicate not significant ($p > .05$; shown for comparison between base and full models). Numbers in parentheses indicate a negative association (reduced symptoms associated with exposure).