Apolipoprotein E genotyping as a potential biomarker for mercury neurotoxicity

Michael E. Godfrey[a,*], Damian P. Wojcik[b] and Cheryl A. Krone[c]
[a]Bay of Plenty Environmental Health Clinic, Tauranga, New Zealand
[b]Northland Environmental Health Clinic, Whangarei, New Zealand
[c]Applied Research Institute, Palmerston North, New Zealand

Abstract. Apolipoprotein-E (apo-E) genotyping has been investigated as an indicator of susceptibility to heavy metal (i.e., lead) neurotoxicity. Moreover, the apo-E epsilon (ε)4 allele is a major risk factor for neurodegenerative conditions, including Alzheimer's disease (AD). A theoretical biochemical basis for this risk factor is discussed herein, supported by data from 400 patients with presumptive mercury-related neuro-psychiatric symptoms and in whom apo-E determinations were made. A statistically relevant shift toward the at-risk apo-E ε4 groups was found in the patients ($p < 0.001$). The patients possessed a mean of 13.7 dental amalgam fillings and 31.5 amalgam surfaces. This far exceeds the number capable of producing the maximum identified tolerable daily intake of mercury from amalgam.

The clinical diagnosis and proof of chronic low-level mercury toxicity has been difficult due to the non-specific nature of the symptoms and signs. Dental amalgam is the greatest source of mercury in the general population and brain, blood and urine mercury levels increase correspondingly with the number of amalgams and amalgam surfaces in the mouth. Confirmation of an elevated body burden of mercury can be made by measuring urinary mercury, after provocation with 2,3,-dimercapto-propane sulfonate (DMPS) and this was measured in 150 patients.

Apo-E genotyping warrants investigation as a clinically useful biomarker for those at increased risk of neuropathology, including AD, when subjected to long-term mercury exposures. Additionally, when clinical findings suggest adverse effects of chronic mercury exposure, a DMPS urine mercury challenge appears to be a simple, inexpensive procedure that provides objective confirmatory evidence. An opportunity could now exist for primary health practitioners to help identify those at greater risk and possibly forestall subsequent neurological deterioration.

Keywords: Apo-lipoprotein E, mercury, dental amalgams, Alzheimer's disease

1. Introduction

Chronic exposure to mercury results in an accumulation in the brain, heart and kidneys, the main target organs [11,30,35]. Dental amalgam has been identified as the largest source of mercury vapor in the non-industrially exposed population [49] and this vapor easily penetrates the central nervous system [34]. Amalgam is uniquely situated in the mouth with mercury having direct access to the olfactory lobes and the limbic brain, via the oro-nasal mucosa and via retrograde axonal transport of mercury, with subsequent preferential accumulation of mercury in those areas [4,19, 31,45,48]. Furthermore, brain mercury levels increase with the number of amalgam fillings or the amalgam "score" that is based on the number of dental surfaces covered in amalgam [31]. A recent US. NIH-funded study of military personnel also confirmed that blood and urine mercury levels corresponded to the number of amalgam fillings, averaging 4.5 times that of controls without amalgam [20]. These authors concluded that amalgam was a major, if not the main, source of mercury found in the body.

*Corresponding author: M.E. Godfrey, Bay of Plenty Environmental Health Clinic, 157 Fraser St. Tauranga, New Zealand. Tel.: +64 7 578 5899; Fax: +64 7 578 2362; E-mail: godfrey@wave.co.nz.

At present, there are no readily acceptable in vivo diagnostic criteria for chronic mercury toxicity; diagnosis is predominantly based on patient history and clinical findings [35]. Long-term exposure to mercury results in central nervous system and other effects. These include: chronic fatigue, irritability, mood swings, poor concentration, mental confusion, chronic headaches, insomnia, and tremors [13,26]. A diagnosis of chronic low-level mercury toxicity, therefore, has been controversial both due to the non-specific nature of the symptoms and signs, and the lack of a reliable in vivo biomarker. However, Apolipoprotein-E (apo-E) genotyping generally utilized in characterization of lipid metabolism, has been studied as a bio-indicator of susceptibility for Alzheimer's Disease (AD) [21,38–40] and a recent paper on apo-E, lead exposure and neurobehavioral function has added support to an association with heavy metal intoxication [44]. A significant relationship was found between the apo-E epsilon (ε)4 allele, impaired cognitive function and susceptibility to toxicity in those previously chronically exposed to lead [44].

Apo-E genotyping determines the inherited parental epsilon 2, 3 or 4 groups, with six homozygous and heterozygous combinations being found (i.e., ε2/2, 2/3, 2/4, 3/3, 3/4 or 4/4). Isomer ε2 has two cysteine amino-acids in its structure, ε3 has one cysteine and one arginine, and ε4 has two arginine amino-acids and no cysteine [6]. Cysteine, with its sulphydryl (-SH) bonds, is potentially able to bind to, and remove metals (e.g., mercury and lead) from tissues, whereas arginine, lacking the -SH bonds, would be unable to do this. Apo-E genotyping therefore becomes relevant once it is acknowledged that prolonged exposure to mercury has been associated with neurotoxicity, including the pathological histology unique to Alzheimer's senile dementia, namely, fibrillary tangles, amyloid plaques and increased phosphorylation of tau protein [12,27,28,32].

A complementary means for detection (and treatment) of heavy metal accumulation is the use of 2,3-dimercaptopropane-sulfonate (DMPS). Although DMPS had previously been extensively reviewed in Soviet literature, it was not until 1983 that a paper by Aposhian [1], detailing the history, pharmacology and uses of DMPS appeared in English. Lead intoxication has been routinely treated for 20 years with the use of DMPS chelation. In 1989, a German toxicologist proposed using DMPS as a diagnostic test for chronic mercury retention and toxicity [9]. Either a 10-fold increase between the pre- and post-challenge urinary mercury levels or a post-challenge level greater than 50micrograms(μg), were suggested as indicators of an abnormally raised mercury body burden [9]. Both intravenous and orally administered DMPS are reported to be effective therapeutic agents [7], and useful in vivo biomarkers [3,9,17,29,42] for mercury accumulation. DMPS is a better biomarker for low-level mercurialism than unchallenged urinary mercury excretion [2,3].

This paper discusses the use of apo-E genotyping and DMPS "challenge" testing as diagnostic adjuvants for identifying those at greater risk of mercury intoxication. We also discuss a theoretical chemical basis for the observation [40] that the apo-E4 allele is a major risk factor for neurodegenerative conditions, including AD.

2. Patients and methods

Patients. Four hundred predominantly Caucasian (>95%) patients (256 female and 144 male) with mean age 50.8 years (range 22–83 years) were seen at two primary health clinics. All patients had histories and or symptoms and signs determined to be suggestive of chronic accumulative exposure to mercury. Patient selection was based on the results of a multi-system questionnaire provided by the International Academy of Oral Medicine and Toxicology (IAOMT, Orlando, Florida). This questionnaire was compiled from symptoms and signs taken from standard toxicology textbooks and the published biomedical literature on mercury toxicity. A scoring system of 0–3 was used to indicate the presence and severity of each individual symptom or sign. A high score was regarded as indicative of likely systemic toxicity. Following a clinical assessment, the patients' dental amalgam status (i.e., total numbers of amalgam fillings and amalgam surfaces) was verified both visually and by dental x-ray. The number of dental surfaces with amalgam or amalgam "score" is a more representative measurement of amalgam mass. Patients were also questioned as to other sources of mercury exposure such as occupational, fish consumption, etc.

Laboratory testing: The potential health relevance of apo-E genotyping was discussed with each patient, and following informed consent being given, the test was included as part of routine hematology and biochemistry investigations. One-hundred fifty patients agreed with written informed consent, to have a urine mercury challenge test to confirm the suspected raised body burden of mercury. A slow (5 minute) intravenous injection of 250 mg DMPS was given after the bladder was emptied and an initial urine sample had been

obtained. Patients then drank approximately 200 ml of water. An aliquot of urine was subsequently taken from the next urine passed 90–120 minutes later. Both samples were then sent in sterile containers for analysis by ICP-Mass Spectrometry at Hills Laboratory, Hamilton, NZ or the Cawthron Institute, Nelson, NZ (both TELARC certified). Five split sample specimens were compared to ensure consistency between laboratories. Urine mercury levels before and after DMPS challenge were determined after adjustment for creatinine.

Statistics. Differences between apo-E patterns in our patients and a control population were compared using Fisher's Exact Test (www.matforsk.no/ola/fisher.htm). Differences were considered significant at $p \leqslant 0.05$.

3. Results

The presenting symptoms in our patient cohort commonly included chronic fatigue, irritability, mood swings, poor concentration, mental confusion, chronic headaches, insomnia, and some tremors, most noticeably of the protruded tongue. Chronic fatigue, the commonest complaint in this cohort of patients, has been confirmed as being associated with hypersensitivity to inorganic mercury and nickel from dental materials, regardless of the underlying disease [43].

A mean of 13.7 (range, 3–24) dental amalgam fillings and a mean of 31.5 surfaces (range, 6–54) were observed in the 400 patients. The apo-E distribution is shown in Table 1. Comparison of our symptomatic cohort ($n = 400$) with blood donors ($n = 426$), using Fisher's Exact Test, revealed a significantly greater proportion of patients exhibiting the $\varepsilon 4/4$ pattern. Moreover, a significantly lower proportion of patients fell into the $\varepsilon 2/2$ and $\varepsilon 2/3$ groups ($p < 0.001$) compared to the blood donors.

Pre-challenge urine mercury levels were invariably low at 5 μg or less mercury/g creatinine. Following the DMPS challenge, a mean of 347 μg of mercury/g creatinine was obtained (range, 30–1852).

Patients were also questioned as to other sources of mercury exposure such as occupational, fish consumption, etc, and no correlation was found with these sources.

4. Discussion

There are three apo-E alleles ($\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$) that are universally distributed in the population with about 30% of the population carrying an $\varepsilon 4$ allele [21,39]. Prior lipid research in New Zealand has indeed confirmed this distribution in New Zealanders with 27.2% of 426 blood donors having the $\varepsilon 4$ isomer and 1% possessing $\varepsilon 4/4$ [46]. Our cohort of patients with suspected mercury-associated symptomology exhibited a shift toward relatively higher proportions of $\varepsilon 4$ isomers. For example, 34% of our subjects exhibited the combined 3/4 and 4/4 isomers compared to 26% found in (presumed healthy) blood donors [46], 23% in healthy Caucasian controls [39], 19% in previously exposed lead workers [44] and 14% in unselected populations [21]. The proportion of homozygous $\varepsilon 4/4$ in our subjects was significantly greater ($p < 0.001$) than in the New Zealand blood donors (3.4% versus 1%). Furthermore, only 9.75% of our cohort had the reportedly AD-protective $\varepsilon 2/2$, $\varepsilon 2/3$ isomers compared to 21.4% in the latter and 17.7% in the general Australian population [25]. Our subjects resembled the relatively lower combined $\varepsilon 2/2$ plus $\varepsilon 2/3$ pattern in symptomatic lead-exposed subjects (10.6% [44]). These results suggest that apo-E genotyping could be used as a biomarker for identifying individuals more prone to experience mercury-related symptoms.

The susceptibility to the long-term effects of lead exposure on the central nervous system (CNS) has been associated with apo-E genotype. Stewart et al. [44] measured tibia bone lead levels and related these to neurobehavioral test scores and apo-E genotype. The authors concluded that the persistent CNS effect of lead may be more toxic in individuals who have at least one apo-E $\varepsilon 4$ allele. To confirm the suspected elevated mercury levels in our cohort of symptomatic subjects, 150 patients consented to undertake DMPS urine mercury challenges. The mean post-challenge mercury level in these patients of 347 μg/g creatinine was 9 times greater than the mean post-challenge mercury level of 39 μg reported in 10 asymptomatic controls (mean age 48 years) never exposed to amalgam [17]. The post-challenge mercury level in our subjects was circa 70 times greater than the pre-challenge mercury ($\sim$5 μg/g creatinine). Aposhian et al. [2] reported a 25-fold increase in urinary mercury of amalgam bearers in the 9 hours after oral DMPS (17.2 μg total mercury excreted) compared to the 9 hours pre-DMPS (0.70 μg). Our higher percentage increase may be due to the rapid excretion following the IV 250 mg DMPS dose and the shorter collection time. If a longer collection is used (i.e., 6 hours), our urinary mercury concentration drops because most DMPS has been excreted within the first 90 minutes [17]. Dental personnel also exhibit raised

Table 1
Distribution of apo-E isomers in mercury-associated symptomatic patients, in New Zealand blood donors [46] and in other adults [25,38,44]

apo-E isomer	Percent possessing isomer (n = number of subjects in study)				
	This study ($n = 400$)	Wardell [46] ($n = 426$)	Roses [38] ($n = ?$)	Stewart [44] ($n = 529$)	Martins [25] ($n = 279$)
2/2	0.25	1.4	1.0	0.2	0
2/3	9.5	20	11	10.4	17.7
2/4	1.2	1.2	5.0	3.2	1.3
3/3	54.8	51.4	60	67.1	59.5
3/4	30.8	25	21	18.0	20.2
4/4	3.5	1.0	2.0	1.1	1.3

Wardell [46], Roses [38] and Martins [25] are healthy control populations and Stewart [44] is a lead-exposed adult population.

urine mercury after oral DMPS (300 mg p.o. after an 11 hour fast) [18]. Six hour urine collections, before and after DMPS, revealed mercury levels and percentage increases generally similar to ours.

Our choice of the intravenous method of DMPS administration has an obvious time advantage for patients traveling from a distance. As with Aposhian et al. [2,3], the purpose of our test was intended to confirm a raised body burden and we were not primarily concerned with the absolute amount of mercury excreted. The test adds laboratory evidence to support the clinical differential diagnosis. Notwithstanding the small numbers so far involved, it was evident that irrespective of the apo-E genotype, those patients with larger amounts of amalgam or who had other metals in the mouth that potentially increased electrolysis, usually had considerably higher post-DMPS urine mercury levels.

According to a 1968 New Zealand Health Department Survey of young people, those 15 years old averaged 13 amalgam fillings and those 21 years old averaged 16 fillings at that time [5]. Changes to dental practice were instigated in 1976, including a directive to cease prophylactic placement of amalgam into non-carious children's teeth. This resulted in a nationwide 64% reduction in amalgam fillings placed over the next 5 years [10] i.e. to < 8 fillings. However, there still remains the now middle-aged cohort of 1.4 million people currently between the ages of 35 and 65 years, out of a population of 3.8 million (NZ population statistics, June, 2000), who have many amalgam filled teeth. The 1996 Health Canada's Risk Assessment on mercury vapor from amalgam, determined that a 70 kg adult would have reached a maximum "tolerable daily intake" (TDI) with 4 average-sized fillings or 8 dental surfaces of amalgam [37]. Based on this TDI, many of the currently middle-aged people with 30 surfaces of dental amalgam, could potentially be at an increased risk of toxicity, including AD. On average, our patients had greater than 3 times the number of fillings and amalgam surfaces that are associated with the maximum TDI. This mercury burden is consistent with the symptom prevalence in our patient cohort.

Although apo-E genotyping has been predominantly directed towards lipid metabolism, it has been identified as a potential biomarker for AD senile dementia, with $\varepsilon2$ being protective and $\varepsilon4$ being predictive at a 70% level [38]. This association has been confirmed for both early and late onset AD in various ethnic populations [21]. However, ethnic variation in AD risk has been observed [21]. For example, in Japanese populations, the association of AD with the $\varepsilon4$ allele is much stronger in Caucasians. And, the AD risk in certain indigenous ethnic populations (e.g., some Africans) is much lower than might be expected from the relatively high frequency of $\varepsilon4$. It has been suggested that lifestyle factors (e.g., diet) may interact with apo-E genotype in raising the risk for $\varepsilon4$-associated disorders [8] such as coronary artery disease (CAD) and Alzheimer's Disease (AD). The absence of the association of $\varepsilon4$ allele with CAD and AD in Sub-Saharan Africans, and its presence in African-Americans is consistent with this hypothesis. When ethnic populations with a high $\varepsilon4$ frequency shift from a traditional diet to the western dietary pattern, the frequency of these disorders increases [8]. We suggest that environmental toxicants, such as mercury from amalgams, could also be considered as a contributor to the changing risk when indigenous populations abandon their traditional, and adopt modern, lifestyles.

According to Saunders, the underlying reason for the apo-E-associated differences in AD susceptibility remains a mystery [40]. However, a logical biochemical explanation has been proposed by Pendergrass and Haley, based on the different amino-acid configurations of the three apo-E isomers and their potential relevance to mercury elimination [33]. Only $\varepsilon2$ (with two cysteine

-SH groups), and to a lesser extent $\varepsilon 3$ (with one -SH group), are able to bind and remove mercury from the brain and cerebrospinal fluid. This would oppose accumulation of mercury which is reported to be causal for the unique brain lesions that typify the AD brain including neuro-fibrillary tangles [22,32,34]. We did not find any evidence of higher post-DMPS mercury excretion in those carrying the $\varepsilon 4$ allele when compared to the other groups. However, this would not be unexpected if mercury from amalgam has direct access to the brain [4,19,31]. Mercury excretion after DMPS challenge reflects a general body burden and especially a renal loading [2,3]. Therefore, a DMPS challenge would not necessarily be a quantitative indicator of brain mercury levels.

Another aspect of AD pathology is the evidence that enhanced mitochondrial damage occurs in AD and $\varepsilon 4$ genotype [16]. Mercury is very destructive at the mitochondrial level where catalase can demethylate organic mercury species into highly reactive inorganic mercury. Inorganic mercury is also an extremely potent enzyme inactivator [47]. Furthermore, chronic micro-mercurial toxicity specifically from dental amalgam has been documented [14,23,31,36,45] and successfully treated by removal of amalgam and medical detoxification in 796 patients [23].

Still, not all research results agree with mercury's causal role in AD. Elevated mercury was not found in seven different regions of AD brains compared to controls [15]. However, the "controls" had possessed three amalgam surfaces whereas the AD subjects had six, likely obscuring any differences. Saxe et al. [41] reporting on the mental health of 129 nuns, found no difference between those with amalgam and controls. However, 72% of the controls had no posterior teeth, and the remainder had a mean of only three teeth. All 129 could, therefore, have had a similar previous amalgam history and the half-life of mercury in the brain is measured in decades. This paper's conclusions, published in a dental trade journal, are at variance with those of another paper in the same journal on risk factors affecting dentists' health. The authors identified 3 factors with equally high statistical values (i.e. $p < 0.001$), namely, a mercury spill in the dental office, manual amalgamation, and the dentists' own amalgam status [24].

Therefore, based on the suggestion that mercury is causal for AD-like lesions due to its unique neurotoxicity, and that $\varepsilon 2$ is potentially protective due to the cysteine amino-acids, it could be contended that: (i) those individuals who have inherited the $\varepsilon 4$ allele and

who are exposed to mercury, including from numerous dental amalgam fillings, would be at greater risk of developing AD at an earlier age than those with the $\varepsilon 2$ configuration; and (ii) those with $\varepsilon 4/4$ would potentially be at the greatest risk of accumulating mercury and developing symptoms. Consistent with this, are our findings that short-term memory loss was common among our patients in the $\varepsilon 4$ groups with only one of those possessing $\varepsilon 4/4$ not listing this as a complaint. Two patients (both $\varepsilon 3/4$) were already suffering from AD and there was a family history of some members having had senile dementia in 20% of the $\varepsilon 3/4$, $4/4$ cohorts.

5. Conclusion

The diagnosis of chronic mercury toxicity has traditionally been based on patient history and clinical acumen, with little reliance on laboratory investigations. The concept of accumulative micro-mercurial neurotoxicity with specific reference to dental amalgam, has been well documented and prolonged exposure to mercury has been associated with the unique lesions of the AD brain. Therefore, amalgam, as the largest source of mercury vapor in the general population, should be included in the differential diagnosis of patients being investigated for neuro-psychiatric problems and short-term memory loss. Apo-E genotyping, a comparatively non-invasive, simple and relatively cheap laboratory test, warrants further investigation as an in vivo biomarker that could support the clinical differential diagnosis in the primary health environment, and help identify those symptomatic patients who are at greater risk of mercury toxicity and of AD. Further supportive evidence of exposure and accumulative retention of mercury, can be obtained from a DMPS-provoked urine mercury challenge.

Acknowledgements

References

[1] H.V. Aposhian, DMSA and DMPS– Water soluble antidotes for heavy metal poisoning, *Ann. Rev. Pharmacol. Toxicol.* **23** (1983), 193–215.

[2] H.V. Aposhian, D.C. Bruce, W. Alter, R.C. Dart, K. Hurl-but et al., Urinary mercury after administration of 2,3-dimercaptopropane-1-sulfonic acid: correlation with dental amalgam score, *FASEB J.* **6**(4) (1992), 2472–2476.

[3] H.V. Aposhian, R.M. Maiorino, D. Gonzales-Ramirez, M. Zuniga-Charles, Z. Xu, et al., Mobilization of heavy metals by newer, therapeutically useful chelating agents, *Toxicology* **97** (1995), 23–38.

[4] B. Arvidson, Accumulation of mercury in brainstem nuclei of mice after retrograde axonal transport, *Acta Neurol. Scand.* **82**(4) (1990), 234–237.

[5] D.R. Beck, Dental Health: Status of the NZ population in late adolescence and young adulthood, *Department of Health Special Report Series* **29** (1968).

[6] D.A. Brouwer, Clinical chemistry of common Apoprotein E isoforms, *J. Chromatography B. Biomed. Applic.* **678**(1) (1996), 23–41.

[7] J.R. Campbell, T.W. Clarkson and M.D. Omar, The therapeutic use of 2,3-dimercaptopropane- sulfonate in two cases of inorganic mercury poisoning, *JAMA* **256** (1986), 3127–3130.

[8] R.M. Corbo and R. Scacchi, Apolipoprotein E (APOE) allele distribution in the world. Is APOE4 a 'thrifty' allele? *Ann. Hum. Genet.* **63**(Pt 4) (1999), 301–310.

[9] M. Daunderer, Mobilisation test for environmental metal poisonings, *Forum des Praktische und Algemende Artzes* **28** (1989), 88–90 (transl).

[10] B. De Liefde, The decline of caries in New Zealand over the past 40 years, *New Zealand Dental Journal* **94** (1998), 109–113.

[11] G. Drasch, E. Wanghofer and G. Roider, Are blood, urine, hair and muscle valid biomonitors for the internal burden of men with the heavy metals mercury, lead and cadmium? *Trace Elements Electrolytes* **14** (1997), 116–123.

[12] E.F. Duhr, J.C. Pendergrass, J.T. Slevin and B.E. Haley, Hg-EDTA Complex inhibits GTP interactions with the E-site of brain beta-tubulin, *Toxicol. Applied Pharmacol.* **122** (1993), 273–288.

[13] D. Echeverria, H.V. Aposhian, J.S. Woods, N.J. Heyer, M.M. Aposhian et al., Neurobehavioral effects from exposure to dental amalgam Hg0: New distinctions between recent exposure and Hg body burden, *FASEB Journal* **12** (1998), 971–980.

[14] J.T.A. Ely, H.H. Fudenberg, R.J. Muirhead, M.G. LaMarche, C.A. Krone et al., Urine mercury in micromercurialism: Bimodal distribution and diagnostic implications Bull, *Environ. Contam. Toxicol.* **63** (1999), 553–559.

[15] Y.K. Fung, A.G. Meade, E.P. Rack and A.J. Blotcky, Brain mercury in neurodegenerative disorders, *J. Toxicol. Clin. Toxicol.* **35** (1997), 49–54.

[16] G.E. Gibson, V. Haroutunian, H. Zhang, L.C. Park, Q Shi et al., Mitochondrial damage in Alzheimer's disease varies with apolipoprotein E genotype, *Ann. Neurol.* **48**(3) (2000), 297–303.

[17] M.E. Godfrey and N. Campbell, Confirmation of mercury retention and toxicity using dimercapto-1-propane-sulphonic acid sodium salt (DMPS), *J. Adv. Med.* **7**(1) (1994), 19–30.

[18] D. Gonzalez-Ramirez, R.M. Maiorino, M. Zuniga-Charles, Z. Xu, K.M. Hurlbut et al., Sodium 2,3-dimercaptopropane-1-sulfonate challenge test for mercury in humans: II. Urinary mercury, porphyrins and neurobehavioral changes of dental workers in Monterrey, *Mexico. J. Pharmacol. Exp. Ther.* **272**(1) (1995), 264–274.

[19] J. Henriksson and H. Tjalve, Uptake of inorganic mercury in the olfactory bulbs via olfactory pathways in rats, *Environ. Res.* **77**(2) (1998), 130–140.

[20] A. Kingman, T. Albertini and L.J. Brown, Mercury concentrations in urine and whole blood associated with amalgam exposure in a U.S. Military population, *J. Dental Res.* **77**(3) (1998), 461–471.

[21] S.M. Laws, E. Hone, S. Gandy and R.N. Martins, Expanding the association between the APOE gene and the risk of Alzheimer's disease: Possible roles for APOE promoter polymorphisms and alterations in APOE transcription, *J. Neurochemistry* (2003), in press.

[22] C.W. Leong, N.I. Syed and F.L. Lorscheider. Retrograde degeneration of neurite membrane structural integrity of nerve growth cones following in vitro exposure to mercury, *NeuroReport* **12** (2001), 733–737.

[23] U. Lindh, R. Hudecek, A. Danersund, S. Eriksson and A. Lindvall, Removal of dental amalgam and other metal alloys supported by antioxidant therapy alleviates symptoms and improves quality of life in patients with amalgam-associated ill health, *Neuroendocrinol. Lett.* **23**(5–6) (2002), 459–482.

[24] M.D. Martin, C. Naleway and H. Chou, Factors contributing to mercury exposure in dentists, *J. Am. Dent. Assoc.* **126** (1995), 1502–1511.

[25] R.N. Martins, R. Clarnette, C. Fisher, G.A. Broe, W.S. Brooks, P. Montgomery and S.E. Gandy, APOE genotypes in Australia: roles in early and late onset Alzheimer's disease and Down syndrome, *Neuroreport* **6** (1995), 1513–1516.

[26] Material Safety Data Sheets (MSDS) and Directions for Use (DFU) Dentsply/Caulk and Ivoclar (Europe), 1997, Available at: http://caulk.com/MSDSDFU/DispersalloyMSDS. Accessed February 1998.

[27] G. McKhan, Clinical diagnosis of Alzheimer's Disease: Report of the NINCDS-ADRA work group, *Neurology* **34** (1984), 929–944.

[28] J. Metuzals, Neuronal transformations in Alzheimer's disease, *Cell Tissue Research* **252** (1988), 239–248.

[29] M. Molin, A. Schutz, S. Skerfving and G. Sallsten, Mobilized mercury in subjects with varying exposure to elemental mercury vapour, *Int. Arch. Occup. Environ. Health* **63** (1991), 187–192.

[30] NIDR/ADA Workshop: Biocompatibility of metals in dentistry, *Journal of American Dental Association (J.A.D.A.)* **109** (1984), 470.

[31] M. Nylander, L. Friberg and B. Lind, Mercury concentrations in the human brain and kidneys in relation to exposure from dental amalgam fillings, *Swedish Dental Journal* **11** (1987),179–187.

[32] G. Olivieri, Ch. Brack, F. Muller-Spahn, H.B. Stahlelin, M. Herrmann et al., Mercury induces cell cytotoxicity and oxidative stress and increases, β-amyloid secretion and tau phosphorylation in SHSY5Y neuroblastoma cells, *J. Neurochem.* **74** (2000), 231–236.

[33] J.C. Pendergrass and B.E. Haley, Mercury-EDTA complex specifically blocks brain β-tubulin-GTP interactions: Similarity to observations in Alzheimer's disease, in: *Status Quo and Perspective of Amalgam and Other Dental Materials, International Symposium Proceedings*, L.T. Friberg and G.N. Schrauzer, eds, Georg Thieme Verlag, Stuttgart-New York, 1995, pp. 98–105.

[34] J.C. Pendergrass, B.E. Haley, M.J. Vimy, S.A. Winfield and F.L. Lorscheider, Mercury vapor inhalation inhibits binding of GTP to tubulin in rat brain: Similarity to a molecular lesion in Alzheimer's disease brain, *Neurotoxicology* **18**(2) (1997), 315–324.

[35] R. Phelps and T. Clarkson, Interrelationship of blood and hair mercury concentrations in a North American population ex-

posed to methyl mercury, *Arch. Env. Health* **35** (1980), 161–165.

[36] J. Pleva, Dental mercury– A public health hazard, *Rev. Environ. Health* **10** (1994), 1–27.

[37] G.M. Richardson and M.A. Allan, Monte Carlo assessment of mercury exposure and risks from dental amalgam, *Human Ecol. Risk Assess.* **2**(4) (1996), 709–761.

[38] A.D. Roses, Apolipoprotein E and Alzheimer's disease. The tip of the susceptibility iceberg, *Annals of N.Y. Academy of Science* **855** (1998), 738–743.

[39] A.D. Roses and A.M. Saunders, Apolipoprotein E genotyping as a diagnostic adjunct for Alzheimer's disease, *Int. Psychogeriatr.* **9**(Suppl.1) (1997), 277–288; discussion 317–321.

[40] A.M. Saunders, Apolipoprotein E and Alzheimer's disease: an update on genetic and functional analyses, *J. Neuropath. Exp.. Neurol.* **59**(9) (2000), 751–758.

[41] S.R. Saxe, D.A. Snowdon, M.W. Wekstein et al., Dental amalgam and cognitive function in older women: findings from the nun study, *J. Am. Dent. Assoc.* **126** (1995), 1495–1501.

[42] R. Schiele, K.H. Schaller and D. Weltle, Mobilization of mercury reserves in the organism by means of DMPS (Dimaval), *Occup. Med. Soc. Med. Prevent. Med.* **24**(11) (1989), 249–251.

[43] I. Sterzl, J. Prochazkova, P. Hrda, J. Bartova, P. Matucha and V.D. Stejskal, Mercury and nickel allergy: risk factors in fatigue and autoimmunity, *Neuroendocrinol Lett.* **20**(3–4) (1999), 221–228.

[44] W.F. Stewart, B.S. Schwartz, D. Simon, K. Kelsey and A.C. Todd, ApoE genotype, past adult lead exposure, and neurobehavioral function, *Environ. Health Perspect.* **110** (2002), 501–505.

[45] C.M. Thompson, W.R. Markesbery, Y.X. Mao and D.E Vance, Regional brain trace element studies in Alzheimer's disease, *Neurotoxicology* **9** (1993), 1–7.

[46] M.R. Wardell, P.A. Suckling and E.D. Janus, Genetic variation in human apolipoprotein E, *J. Lipid Res.* **23**(8) (1982), 1174–1182.

[47] J.L. Webb, *Enzyme and metabolic inhibitors*, Academic Press, New York, 1963.

[48] D. Wenstrup, W.D. Ehmann and W.R. Markesbery, Trace element imbalances in isolated sub-cellular fractions of Alzheimer's disease brains, *Brain Research* **553** (1990), 125–131.

[49] *World Health Organisation: Environmental Health Criteria* **118** (1991), 74.