DIPLOMARBEIT

Titel der Diplomarbeit

„The relevance of the individual genetic background on prenatal mercury exposure in Bratislava“

verfasst von

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angestrebter akademischer Grad

Magistra der Naturwissenschaften (Mag.rer.nat)

Wien, 2013

Studienkennzahl lt. Studienblatt:	A 442
Studienrichtung lt. Studienblatt:	Diplomstudium Anthropologie
Betreuerin:	Assoc. Prof. Priv. Doz^{in}. Mag.^{a} Dr.^{in} Claudia Gundacker

Aufgrund des von mir gewählten Doppelstudiums Anthropologie und Ökologie in der Studienrichtung Biologie, entstanden zwei von mir verfasste Diplomarbeiten. Beide Diplomarbeiten („*The influence of diet in prenatal mercury exposure in Bratislava and Vienna“* und „The relevance of the individual genetic background on prenatal mercury exposure in Bratislava“) befassen sich mit der pränatalen Quecksilberbelastung (mit unterschiedlichen Schwerpunkten), daher kann es sowohl zu inhaltlichen als auch zu sprachlichen Überschneidungen kommen.

Danksagung

An erster Stelle möchte ich mich bei meiner Betreuerin Assoc. Prof. Priv. Doz [in]. Mag.[a] Dr.[in] Claudia Gundacker bedanken. Zum einen dafür, dass sie mich mit dem Thema dieser Diplomarbeit bekannt gemacht hat und zum anderen für die großartige Betreuung, Unterstützung und Geduld während meines Schreibprozesses. Weiters möchte ich mich auch bei Matthias Scheinast für die geduldige Unterstützung während der praktischen Arbeit bedanken.

Mein besonderer Dank gilt auch meiner persönlichen SPSS Notrufhotline Mag[a]. Alexandra Mühlhauser, für ihre Geduld und stets den richtigen Vorschlägen, damit meine Vorstellung einer Grafik mit der vom SPSS generierter Grafik übereinstimmt, die doch sehr oft weit auseinander lagen. Zusätzlich möchte ich auch bei Mag[a] Nika Pende, Maria Moffat und Mag[a]. Julia Zerelles für das sprachliche Feedback bedanken. Ohne euch vier, hätte mir das Biologiestudium nur halb so viel Spass gemacht.

Ein weiter Dank gilt Danae Pifeas, die bei unseren wöchentlichen Chai-Latte Päuschen immer ein offenes Ohr für die Höhen und Tiefen meiner Diplomarbeit hatte.

Mein größter Dank gilt meiner Mutter Ulrike, die mich von klein auf frei entscheiden hat lassen, welchen Bildungsweg ich einschlagen möchte und bei all meinen Entscheidungen beratend und unterstützend an meiner Seite stand. Ohne sie und ihr stetiges Vertrauen mir gegenüber hätte ich es nicht bis hierher geschafft. Ein großer Dank gilt auch meiner groß(artig)en Schwester Annamaria, die mir gerade in den Anfängen des Studiums eine sehr große Hilfe war und für mich - egal was bei ihr gerade los war- immer ein starker Halt war um aus der ein oder anderen Krise raus zu finden und mir somit die Kraft gab mich auf mein Studium und Diplomarbeit zu konzentrieren.

Abstract

The heavy metal mercury (Hg) is a ubiquitous environmental toxicant. Prenatal exposure can lead to neurological disorders. This neurotoxin is able to cross the blood-brain barrier as well as the placenta barrier. Especially during pregnancy and early childhood, Methyl-Hg exposure can lead to impairment of neurological development. Epidemiological studies on long-term effects of chronic Hg exposure in New Zealand, Faroe Islands or the Seychelles, showed inconsistent results. This might be caused by variable lifestyle, diet and by genetic background. There is increasing evidence that certain genotypes modify the Hg metabolism. The aim of the study is to determine the Hg exposure in Bratislava and to identify a potential association between Hg exposure and polymorphisms in candidate genes assumed to be involved in Hg toxicokinetics.

Study design: 100 Mother-Child-Pairs were recruited at the Ruzinov clinic in Bratislava. The study participants were interviewed about potential exposure factors. Maternal blood and cord blood samples were collected around gestational week 36 and directly after birth, respectively. Hg concentrations were analyzed in the erythrocyte fraction of maternal blood (MatBl-Ery-Hg) and cord blood (CordBl-Ery-Hg) by CV-AFS. 16 SNPs of interest were examined by TaqMan method. The modulators of Hg exposure were determined by bivariate statistical analysis and categorical regression analysis (CATREG).

Results: The median Hg concentration in MatBl-Ery was 1.56 µg/kg (range 0.51-4.58 µg/kg) and in CordBl-Ery 2.05 µg/kg (range 1.03-6.93 µg/kg) respectively. The variant carriers of certain *GSTP1-105* (rs1659) and *ABCC2* (rs3740066) polymorphisms had higher Ery-Hg concentrations compared to the carriers of the common allele. Variations in the *ABCB1* gene (rs1045642, rs1128503, rs2032582) were significantly associated with lower Ery-Hg levels.

Conclusion: The mean Hg body burden of our study participants was low. Furthermore, we found evidence that certain genotypes influence the Hg toxicokinetics in humans.

Zusammenfassung

Das Schwermetall Quecksilber (Hg) ist ein weitverbreiteter Schadstoff. Da das Neurotoxin Hg die Fähigkeit hat, sowohl die Blut-Hirnschranke als auch die Plazenta zu passieren, kann es gerade während der Schwangerschaft zu Störungen in der neuronalen Entwicklung des Fötus kommen. Epidemiologische Studien, die auf den Seychellen, Färöer Inseln und Neuseeland durchgeführt wurden, zeigten widersprüchliche Ergebnisse über die Langzeitfolgen chronischer Quecksilberbelastungen. Diese Unstimmigkeiten können auf unterschiedliche Ernährung, den Lebensstil oder genetische Veranlagung zurückzuführen sein. Frühere Forschungsergebnisse legen nahe, dass bestimmte Polymorphismen in Genen, die in den Hg-Metabolismus involviert sind, die Hg-Belastungen modulieren können. Ziel dieser Studie war, die aktuelle Hg-Belastung im Ballungsraum Bratislava festzustellen. Weiters stellte sich die Frage inwiefern der genetische Hintergrund von Mutter und Kind einen Einfluss auf die jeweilige Hg-Belastung hat.

Studiendesign: In der Ruzinov Klinik in Bratislava (Slowakei) wurden 100 schwangere Probandinnen rekrutiert. Einflussfaktoren auf den Hg-Gehalt wurden mittels Fragebogen erhoben. Der Hg-Gehalt wurde mittels CV-AFS in der Erythrozytenfraktion im Blut der Mutter (MATBL-Ery-Hg) bzw. im Nabelschnurblut des Kindes (NAB-Ery-Hg) bestimmt. Die Polymorphismen in ausgewählten Genen von Müttern und Kindern wurden mittels TaqMan-Methode genotypisiert. Die relevanten Einflussfaktoren (Lebensstil, Ernährung bzw. genetischer Hintergrund) der Hg-Gehalte in Müttern und Kindern wurden mittels bivariater statischer Analyse und kategorialer Regression ermittelt.

Ergebnisse: Die Untersuchungen ergaben einen Median der Hg-Konzentration im maternalen Blut von 1.56 µg/kg (0.51-4.58 µg/kg) bzw. von 2.05 µg/kg im Nabelschnurblut (1.03-6.93µg/kg). Weiters zeigte sich, dass Träger/innen bestimmter Varianzallele [z.B. *GSTP1-105 (*rs1659) und *ABCC2* (rs3740066)] höhere Hg-Werte im Blut haben verglichen zum Wildtyp. Bestimmte Sequenzvariationen im *ABCB1* Gen (rs1045642, rs1128503, rs2032582) hingegen waren mit niedrigeren Hg-Werten im Blut assoziert.

Conclusio: Die durchschnittliche Hg-Belastung der hier untersuchten Probanden ist nicht besorgniserregend. Desweiteren konnte ein Zusammenhang zwischen dem Vorhandensein bestimmter Single Nukleotid Polymorphismen (SNPs) und der Hg-Belastung im menschlichen Organismus aufgezeigt werden.

Table of contents

Abbreviation 1

List of tables and figures 3

Tables 3

Figures 3

1I Introduction 5

1I1 Inorganic Hg 5

1I2 Organic Hg 6

1I3 Biochemical Hg cycle 6

1I4 Toxicity of Hg: Human epidemiological data 7

1I5 Molecular mechanism of MeHg-induced neurotoxicity 9

1I6 Deposition of Hg in humans 10

1I7 Uptake and Efflux of MeHg based on cellular mechanism 11

1I7I1 Glutathione (GSH) system related enzymes 12

1I7I1I1 γ -glytamylcysteine-synthetase (GCL) 12

1I7I1I2 Glutathione-S-transferase (GSTs) 13

1I7I2 Metallothioneins (MTs) 13

1I7I3 ATP binding cassettes transporter proteins (ABC transporter) 14

1I7I3I1 *ABCB1* 14

1I7I3I2 *ABCC1* 15

1I7I3I3 ABCC2 15

1I8 Candidate genes 15

1I9 The aims of the study 17

2I Materials and Methods 19

2I1Sampling 19

2I2 Preparation and acid digestion of samples 20

2I3 Analysis of Hg.. 21
2I3 Genetic analyses 23
2I4 Statistical analyses 26
3I Results 29
3I1 Hg concentrations in maternal blood and cord blood.................................. 29
3I2 Determinants of Hg exposure 29
3I3 Genetic influence on Hg levels of mother-child-pairs.................................. 33
4I Discussion.. 39
4I1 Non-genetic factors related to Hg body burden 40
4I2 Genetic factors related to Hg body burden 41
4I2I1 *GSTP1* and Hg exposure 42
4I2I2 *ABCB1* and Hg exposure 42
4I2I3 ABCC2 and Hg exposure 44
5I Conclusion 45
6I References... 47
7I Appendix 53
8I Curriculum Vitae... 65

Abbreviation

ABC transporter	ATP binding cassettes transporter
ATP	Adenosine triphosphate
CATREG	Categorical regression analysis
CNS	Central nervous system
CordBl-Ery-Hg	Cord blood erythrocyte mercury
NAB-Ery-Hg	Nabelschnurblut Erythrozyten Quecksilber
CV-AFS	Cold vapor atomic fluorescence spectroscopy
dH_20	Millipore water
Ery	Erythrocytes
EtHg	Ethyl mercury
GCL	γ -glytamylcysteine-synthetase
GR	Glutathione reductase
GS	Glutathione synthetase
GSH	Glutathione
GST	Glutathione-S-transferase
GPx	Glutathione peroxidase
HBM	Human BioMonitoring
Hg	Mercury
Hg^0	Elemental mercury (vapor or liquid)
Hg^{2+}	Mercuric mercury
kDA	KiloDalton
MatBl-Ery-Hg	Maternal blood erythrocyte mercury
MatHair-Hg	Maternal hair mercury
MeHg	Methyl mercury
MDR	Multidrug resistance proteins
mRNA	messenger Ribonucleic acid
MRP	Multidrug resistance associated proteins
MSD	Membrane spanning domain
MT	Metallothionein
NBD	Nucleotide binding domains
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain reaction–restriction fragment length polymorphism
P-gp	P-glycoprotein
PTWI	Provisional tolerable weekly intake
qRT-PCR	Real time polymerase chain reaction
ROS	Reactive oxygen species

SNP	Single Nucleotid Polymorphism
WHO	World Health Organization

List of tables and figures

Tables

Table 1. Detailed information on selected candidate genes
Table 2. Profile of the study group
Table 3. Program for digestion of blood samples in microwave mls 1200 mega
Table 4. PCR protocol of *MT, GSTP1, GCLC, GCLM, ABCB1* and *ABCC2* genotypes analysis
Table 5. Primer and digestion protocol of *MT*, *GCLM* and *ABCC1* genotypes PCR-RFLP analysis
Table 6. Categorization of metric variables
Table 7. CATREG models of MatBl-Ery-Hg
Table 8. CATREG models of CordBl-Ery-Hg
Table 9. Hg concentrations in maternal and cord blood
Table 10. Correlation of Ery-Hg levels and non-genetic factors
Table 11. Candidate genes and genotype frequencies
Table 12. Independent determinants of MatBl-Ery-Hg (CATREG analysis)
Table 13. Independent determinants of CordBl-Ery-Hg (CATREG analysis)
Table 14. Comparison of selected data on Hg levels in maternal and umbilical cord blood
Table 15. Current data on the functional impact of *ABCB1* polymorphisms

Figures

Figure 1. Global cycle of Hg [1]
Figure 2. Scheme of MeHg-induced oxidative stress, Ca^{2+} and gluatamate dyhoemostasis [2]
Figure 3. Hg distribution and depostition in the body. [3]
Figure 4. The major common cellular mechanism of uptake and effluxe of Hg © C.Gundacker
Figure 5. Mercur plus, Analytik Jena [4]
Figure 6. Schematic diagram of Hg analysis by AFS [4]
Figure 7A. MatBl-Ery-Hg levels in relation to fish consumption
Figure 7B. Association between CordBl-Ery-Hg concentration and fish consumption
Figure 7C. Total fish consumption in dependence of maternal education
Figure 8A. MatBl-Ery-Hg levels in relation to dental amalgam fillings
Figure 8B. Fish consumption habits in groups with different numbers of amalgam fillings
Figure 8C. Sex-specifc CordBl-Ery-Hg contents
Figure 8D. Cross-tabulation of the variables fish consumption and dental amalgam fillings
Figure 8E. Cross-tabulation of fish consumption and sex of the child,
Figure 9. MatBl-Ery-Hg concentration in dependence on *ABCB1* (rs1045642) genotype
Figure 10A. CordBl-Ery-Hg levels in dependence on maternal *ABCB1* (rs1128503) genotype

Figure 10B. CordBl-Ery-Hg concentration in dependence on maternal *ABCB1 (*rs203258) genotype

Figure 10C. CordBl-Ery-Hg levels in dependence on maternal *GSTP1-105* (rs1659) genotype

Figure 10D. CordBl-Ery-Hg level in dependence on children *ABCC2* genotype (rs3740066)

1I Introduction

The non-essential heavy metal mercury (Hg) is highly toxic for humans. The characteristic of this silver, shiny heavy metal is to be ubiquitous with high potential for bioaccumulation. Moreover, it has the ability to cross the blood-brain barrier, as well as the placenta barrier. Mainly inorganic elemental mercury (Hg^0) and organic methylmercury (MeHg) and ethyl mercury (EtHg) have an impact on the human body. Inorganic and organic Hg vary in their toxicological properties. The main source of Hg burden is fish consumption (source of MeHg), dental amalgam fillings (source of Hg^0) and medical products (source EtHg) [3].

1I1 Inorganic Hg

Elemental mercury (Hg^0), mercurous mercury ($Hg\text{-}Hg^{2+}$) and mercuric mercury (Hg^2) belong to the group of inorganic Hg. One characteristic of Hg^0 is to be liquid at room temperature and when room temperature rises, the liquid Hg^0 is going to evaporate in the form of Hg^0 vapor. Hg^0 is used in dental amalgam fillings, which contain metallic mercury to about 50%. Dental amalgam fillings continuously release a small amount of Hg^0 vapor, which is caused by chewing, tooth brushing or drinking hot beverages. The released Hg^0 vapor is then either absorbed through the oral mucosa or inhaled. Furthermore, fluorescent light bulbs contain Hg^0, which does not pose a risk unless bulbs are broken or damaged, and Hg^0 vapor is released.[3] In the gold mining industry, Hg^0 is an essential requisite, where gold extraction by amalgamation is a very common method. By burning the amalgam, Hg^0 is evaporated and only gold is left over. During this procedure a high amount of Hg^0 is released, which can be inhaled by gold miners. [5] Almost 80% of an inhaled Hg^0 vapor dose is absorbed through the lungs. In the lungs, the vapor can cross the cell membrane and diffuse into the cardiovascular system. Hg^0 binds on the erythrocytes in the blood to reach the target organs, kidneys and brain. Hg^0 accumulates mainly in kidneys and induces dysfunction in the proximal tubule in primary urine resorption, which can cause proteinuria. The kidneys are the main target organ of Hg^0. Exposure of Hg^0 vapor also has a limited capacity of crossing the blood-brain barrier and can accumulate in brain tissue. Basically, Hg^0 has a half-life between 35-90 days in the human body and will be excreted by urine [3, 6].

1I2 Organic Hg

Organic Hg compounds include ethyl mercury (EtHg), also called thiomersal, and methylmercury (MeHg). Thiomersal is widely used as a preservative in vaccines and cosmetic products. Until 1970's EtHg was also used as fungicide. Based on a short half-life of 2-8 days and the low content in vaccines or other medical products (0.001%-0.01%), it has been concluded that EtHg does not substantially influence Hg exposure in humans. Compared to EtHg, MeHg is the common form of organic Hg to which the human body is exposed, basically due to fish consumption. The half-life of MeHg ranges between 50-90 days. The main target organ of MeHg is the brain and the central nervous system (CNS) [3]. It can cause neuronal degeneration by affecting the microtubule integrity and can lead to disarrangement of neuronal migration and disturbance in nervous conduction of impulses and cytoarchitecture in the brain. Additionally, MeHg may lead to depolymerization of existing microtubules, demyelination and loss of granule cells and motor neurons [7-9]. The neuronal cells seem to be more affected by MeHg although it mainly accumulates in glial cells in the brain [10]. The vulnerability of cells in the CNS depends on its repair or protective mechanisms against MeHg (as reviewed by Clarkson [11]).

1I3 Biochemical Hg cycle

Hg is continually cycled and recycled in the environment (Fig. 1) The natural Hg emissions, caused by volcano activity, forest fires and erosion of rocks or soils. Those have a mild impact on the global Hg pollution compared to the anthropogenic associated emission (coal-fired power plants, fossil fuels burning, gold mining and the use of Hg containing products such as light bulbs) [6]. Based on the high impact of the anthropogenic factors, the Hg exposure has been tripled over the last 150 years [12]. The emitted Hg^0 vapor remains in the atmosphere for years and covers huge distances. While it remains in the atmosphere, Hg^0 is slowly converted to Hg^{2+}, which returns to the earth's surface by rainwater. If it reaches the water surface, Hg^{2+} goes through the process of downwelling to the aquatic sediment, where microorganisms, mainly sulfate-reducing bacteria, convert Hg^{2+} to MeHg. This process, which is called biomethylation, is influenced by temperature, pH and salinity of the water. Biomethylation of Hg^{2+} could be interpreted as a protective measure for microorganisms, because Hg^{2+} is more toxic to them than MeHg. The dissolved

MeHg is released to the water column, where it crosses the biological membrane of phytoplankton and subsequently enters the food chain. At each trophic level of the food web, MeHg undergoes a biomagnification process. That is why predatory fishes like tuna, shark or swordfish have higher Hg levels than lower trophic level fishes like salmon, anchovies, etc. [13].

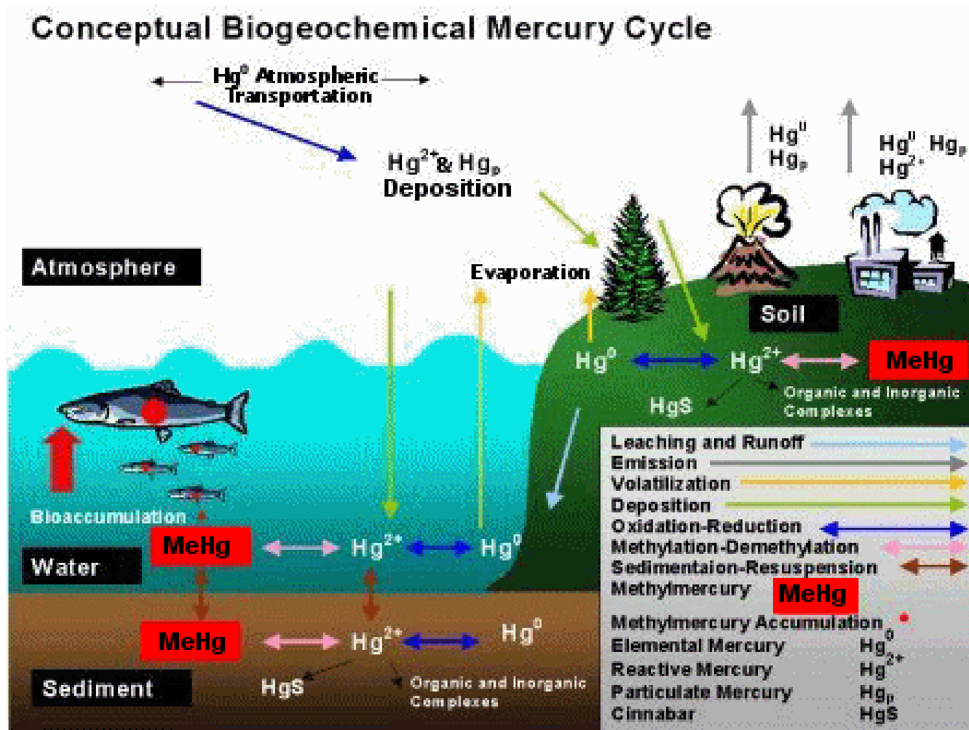


Figure1. Global cycle of Hg [1]

1I4 Toxicity of Hg: Human epidemiological data

Since the mass-poisoning in Minamata, Japan, in the early 1950s, the toxicity of Hg is known. The chemical factory Chisso Cooperation used inorganic Hg for the production of acetataldehyd, which produces MeHg as a side product. Over the years, the company released waste water, contaminated with MeHg, to Minamata bay, which led to high levels of Hg in fish and sea food [3]. By consuming highly contaminated fish and sea food over years, people developed symptoms of Hg intoxication like ataxia, tremor, constriction of visual fields and disturbance in sensory functions. These symptoms are caused by neuronal degeneration in the auditory, motor, sensory and visual areas of the brain [14]. During the Minamata outbreak, Hg blood levels above 200 µg/L of affected people have been observed. Moreover, it

was found that MeHg passes the placenta barrier, as well as the fetal blood-brain barrier, due to the fact that pregnant women with no or mild symptoms of Hg poisoning gave birth to babies with much more pronounced symptoms like mental retardation, blindness and cerebral palsy. The fetus is more sensitive to this neurotoxin than mothers because of its immature detoxification system and rapid metabolism. Furthermore MeHg-induced toxicity depends on the period of exposure due to the fact that maturing organs are more vulnerable to perturbation than after complete maturation. In a meta-analysis it was also shown that Hg blood levels of the newborns are higher than the observed blood levels of mothers [15-17]. Based on this mass poisoning in Minamata, the chronic MeHg exposure through fish consumption was investigated by three major epidemiological studies on the Seychelles, the Faroe Islands and in New Zealand [3]. The aim of these studies was to monitor the cognitive abilities and fine motor skills of children in different ages in association with their prenatal Hg body burden. Because of the geographic area and culture, all three cohorts differed in amount and species of their regular fish consumption. The Seychellois consume a high amount of ocean fish on a daily basis, while the Faroese prefer to eat whale meat, especially blubber, on an irregular basis. The third study was performed in a New Zealand population with a low consumption of fish. The results of these epidemiological studies varied significantly. The results of study participants in the Seychelles cohort indicated no association between prenatal Hg exposure and deficits in cognitive skills or fine motoric abilities. While the results of the Faroe Islands cohort showed that there is a relation between CordBl-Hg-Level above 50 µg/L and some cognitive and motoric deficits [18]. It was entirely unexpected that the mean maternal hair level (MatHair-Hg level) of the Faroese (4.8 ppm) was lower than the monitored average MatHair-Hg level of the Seychellois (6.1 ppm). In New Zealand, children with a high prenatal Hg exposure (above 6 ppm Mat-Hair-Hg level) had lower scores in neurological tests compared to children with a lower prenatal Hg exposure [3]. Further studies showed that Hg blood levels between 5 and 190 µg/L could lead to symptoms like deficits in attention, language, memory and fine motor skills or mental retardation and developmental delay. The severity of the mentioned symptoms depended on the Hg body burden [19]. The "no observed adverse effect level" (NOAEL) is still unknown. However, the World health

organization (WHO) recommended a provisional tolerable weekly intake (PTWI) of 1.6 µg MeHg/kg body weight [20]. Furthermore, the European Union allows only 0.5 mg/kg Hg content in fish except highly contaminated fishes like predatory fishes (tuna, shark and swordfish) with a Hg content of 1 mg/kg. These limit values of Hg may help to minimize the Hg body burden through fish consumption [21].

1I5 Molecular mechanism of MeHg-induced neurotoxicity

Astrocytes represent 50% of the CNS and regulate the extracellular ion concentration, the extracellular pH and the uptake of neurotransmitters, i.e. glutamate. Astrocytes are essential for the synthesis and elaboration of cues for neuronal migration during brain development. They also produce neurotrophic factors, which are essential for neuronal differentiation and division. MeHg, which accumulates in astrocytes, can cause an inhibition of cysteine and cystine transport and can therefore adversely influence the glutathione (GSH) content and its redox status. In astroctyes, MeHg can cause an inhibition in the glutamate uptake and concomitantly it stimulates the efflux of glutamate. Consequently, the extracellular glutamate level in the synaptic cleft increases (reviewed by Aschner et al. [22]). Glutamate is an excitatory neurotransmitter and plays a key role in memory, learning and neuronal development [23]. High concentration of extracellular glutamate can cause excitotoxic injuries in neural cells. It induces overactivation of the N-methyl D-aspartate (NMDA) type glutamate receptors, which leads to an increased influx of Na^{+} and Ca^{2+} into the neurons. Increased Ca^{2+} concentration in the neuron leads to relocation of the Ca^{2+} in the mitochondria and generates an increased level of reactive oxygen species (ROS) or directly activates the cell death pathway. Furthermore, MeHg can directly generate increased levels of ROS, which adversely influences the glutamate uptake in the astrocytes (see Figure 2, reviewed by Farina et. al [2]).

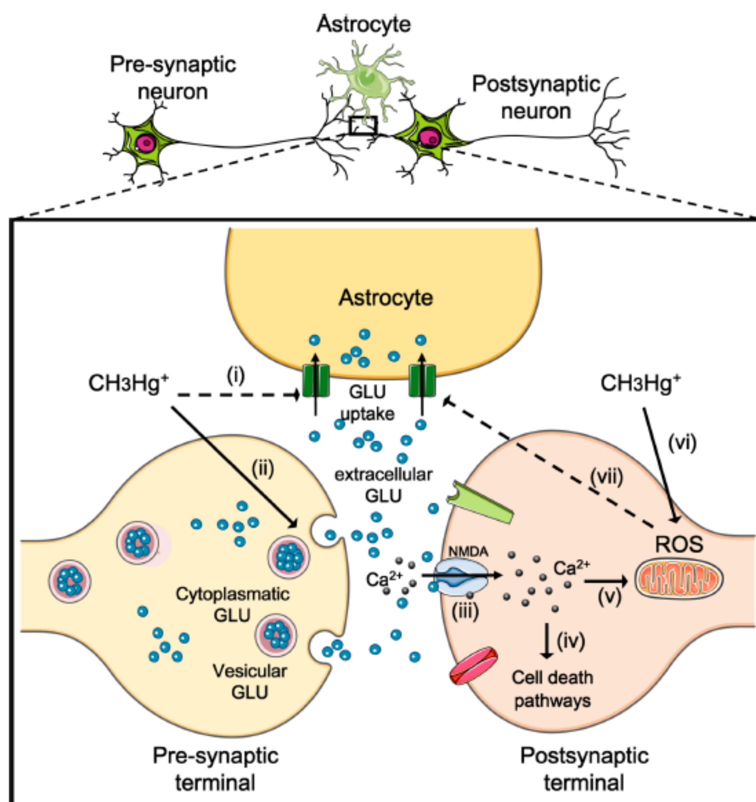


Figure 2. Scheme of MeHg-induced oxidative stress, Ca^{2+} and gluatamate dyhoemostasis [2]

In summary, the MeHg-induced neurotoxicity includes alterations of receptor and transporter activity (including neurotransmitters), intercellular calcium dyhoemostasis, disturbance in the glutamate metabolism and oxidative stress aggravation (reviewed by Liu et al [24]).

1I6 Deposition of Hg in humans

The intestinal tract absorbs almost 95% of MeHg in contaminated food, which is then transported to the liver. The MeHg binds to the thiol group of cysteine. The emerging complex is able to cross cell membranes. Hg^{2+} is intracellularly conjugated to GSH, which effluxes it from the cell. Based on this mechanism (see section 1I7), MeHg can easily be transported through the body. MeHg forms a complex with reduced GSH in liver cells, which is subsequently excreted from liver cells to the bile by GSH carriers [3]. In the bile, enzymes hydrolyzed this GSH complex into its 3 components: glutamic acid, glycine and cysteine (as a cysteine-MeHg complex). This cysteine-MeHg-complex is partly reabsorbed into the blood stream and transported to the

brain, the kidneys or in case of a pregnancy, the fetus. If this complex is not reabsorbed in the blood stream, it is secreted to the intestinal tract, where it is either reabsorbed into the blood stream or slowly demethylated to Hg^{2+} by microorganisms of the intestinal tract and then excreted by feces (see Fig. 3). In the blood stream, MeHg binds to the cysteinyl residues of the hemoglobin. That is the reason why the concentration of MeHg is 20 times higher in erythrocytes than in plasma [11]. It takes about 30 hours for the absorbed MeHg to disperse in the body, whereof 1% is excreted per day [3].

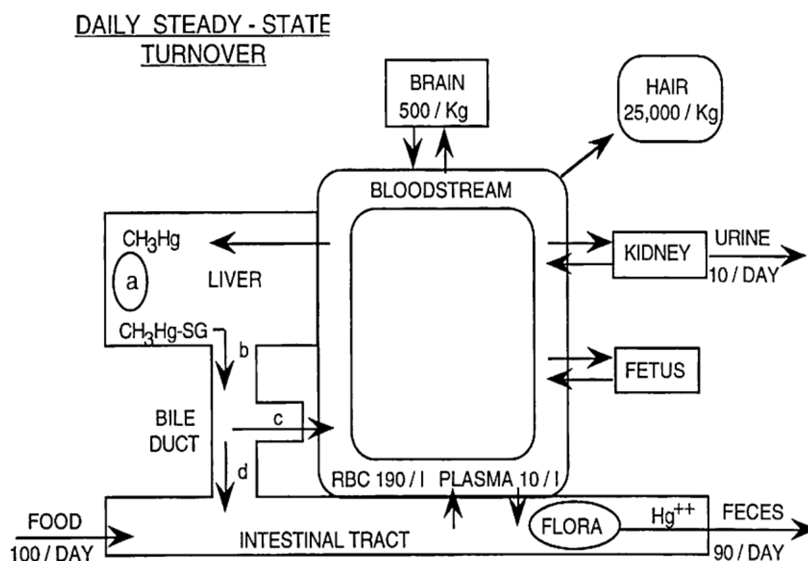


Figure 3. Hg distribution and depostition in the body. [3]

1I7 Uptake and Efflux of MeHg based on cellular mechanism

Hg^0 vapor crosses the cell mebrane by passive diffusion, whereas the (Me)Hg, which is bound to cystein, crosses the cell membrane by active transport through L-type amino acid transporters (LATs) or the organic anion transporters (OATs). In the cell, different forms of Hg are metabolized to Hg^{2+}. Hg^{2+} has a high affinity to thiol groups and conjugates to the tripeptide GSH, catalysed by the enzyme glutathione-S-transferase (GST). GSH is known as a major intracellular antioxidant and conjugation agent. This GSH-Hg complex is effluxed from the cell by ATP-binding cassette transporter proteins (ABC transporter). In addition to GSH, Hg^{2+} is also able to bind on the proteins metallothioneins (MTs), which store the Hg^{2+} in the cell (Fig.4) [25-28].

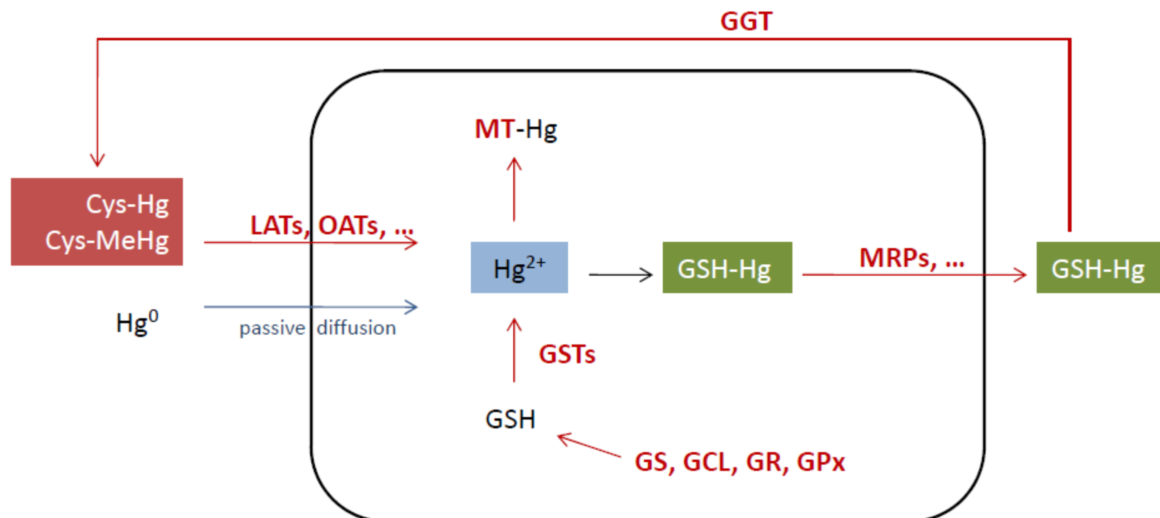


Figure 4. The major common cellular mechanism of uptake and effluxe of Hg © C.Gundacker

1I7I1 Glutathione (GSH) system related enzymes

With the assistance of the enzyme γ -glytamylcysteine-synthetase (GCL) and ATP, cysteine and glutamic acid form the complex of γ -glutamylcysteine. GCL is the limiting factor of the GSH biosynthesis. The γ -glutamylcysteine is then converted to GSH in the presence of glycine, the enzyme GSH-synthetase (GS) and ATP. The enzymes glutathione reductase (GR) and glutathione peroxidase (GPx) work in concert to maintain that enough GSH in its reduced form is available. Only the reduced form of GSH is able to bind to Hg^{2+}. Another important enzyme is glutathione-S-transferase (GST), which catalyzes conjugation of xenobiotics or other substrates (including endogenous substrates) to reduced GSH. The GSH conjugation rate of Hg depends on interaction of all GSH system components. The resulting complex can be easily eliminated from the cell by multidrug-resistance proteins (MRPs), most likely via MRP2, a member of the ABC transporter superfamily [26, 27].

1I7I1I1 γ -glytamylcysteine-synthetase (GCL)

The GCL enzyme contains two subunits, the catalytic GCLC subunit and GCLM, the modifying unit. The catalytic unit is responsible for all catalytic activity of the holoenzyme. The modifying unit has no enzymatic activity, but rather a regulatory function by increasing the affinity of GCL for glutamate. A C/T substitution at position

129 of GCLC (rs17883901; located at 6p12) results in a decreased rate of producing GSH. The same effect was observed in *GCLM 877* (rs41303970; located 1p22.1) [26, 29].

1I7I1I2 Glutathione-S-transferase (GSTs)
The enzyme GSTs catalyzes the binding reaction with GSH and xenobiotics or (endogenous) substrates and leads to a stable GSH complex. GST is represented in six cytosolic subclasses.

GSTM1 (Mu Class of GST; located at 1p13.3) and *GSTT1* (Theta class; located at 22q11) show a deletion polymorphism, which causes an impairment of the catalytic activity. Carriers of these polymorphisms exhibit no or less enzyme activity, which causes lower levels of GSH-conjugate followed by a reduced elimination rate. [30]

Two different single nucleotide polymorphisms (SNPs) occur in GSTP1 (Pi class; located at 11q13), *GSTP1-105* (rs1695; Ile105Val) and *GSTP1-114* (rs1138272; Ala114Val), which have a direct impact on the enzyme activity of GSTP1. The amino acid substitution of *GSTP1-105* affects the geometry of the substrate binding site of GSTP1, while the amino acid substitution of *GSTP1-114* may have an influence on the activity of this enzyme [31].

1I7I2 Metallothioneins (MTs)
MTs are 6-7 kDa small proteins, which are rich in cysteine. It is noteworthy that almost 30% of the protein are cysteines. With this property, the four isoforms of MTs are qualified to bind and detoxify essential and non-essential metals, predominantly zinc, copper, and cadmium. They are located in the golgi apparatus of the cell. Moreover MT1-4 play an important role in protecting against oxidative stress by adapting to a variety of stressors, whereas MT1-MT3 are also known to have a protective function in the brain [26, 28].

1.7.3 ATP binding cassettes transporter proteins (ABC transporter)

ABC transporters are one of the largest protein groups and can be found in every kind of organism. The ABC transporters are subdivided in seven subfamilies (A-G). This work only focuses on ABCB and ABCC transporter, especially on *ABCB1*, *ABCC1* and *ABCC2*. ABC transporters are membrane proteins, which translocate a huge variety of different substances to various intracellular compartments or export it from the cell [26]. These membrane proteins consist of two polytrophic membrane spanning domains (MSDs) and two nucleotide binding domains (NBDs). The NBDs include the Walker A, as well as the Walker B motif, which are essential for ATP binding and hydrolysis. In addition to that the ABC transporter also contains a C-motif, which is necessary for substrate binding and also for the hydrolysis of ATP [32]. The underlying transport mechanism is that the ATP-dependent closure/ dimerization of cytosolic NBDs regulates the power stroke that pulls the TMDs from an inward- to outward-facing conformation and shifts the transported substrate into a position, where it can be released from the cell [33]. Based on these properties the ABC transporters are classified as active transporters, that are able to transport against a concentration gradient [34].

1.7.3.1 *ABCB1*

ABCB1, also called Multidrug resistance protein 1 (MDR1) or P-glycoprotein, is located at 7q21. This 170kDa glycoprotein is located at the apical surface of the epithelial cells and it is highly present at the blood-brain barrier and the placenta barrier [35]. P-glycoprotein influences the uptake of substances at the blood-brain barrier. A high expression in this area might decrease the uptake rate of xenobiotics into the brain, while a reduced expression leads to a higher accumulation rate [36]. *ABCB1* has the typical four domain structure of the ABC transporters: two NBDs precede by MSDs, which contain six transmembrane (TM) helices [37]. Allelic differences in *ABCB1* gene sequences influence the expression level or alter the substrate-binding spectrum [36]. For the *ABCB1* gene, more than 50 SNPs have been reported; one of them is *ABCB1* rs1045642, located at exon 26, which is associated with altered transport activity of *ABCB1* [38].

1I7I3I2 *ABCC1*

ABCC1, which encodes the 190kDa Multidrug resistance-associated protein 1 (MRP1), is located at 16q13.12-13. It is highly expressed in lung, testis, kidney and blood mononuclear cells, while it is less expressed in the liver. MRP1 is mainly located in the basolateral cellular surface. Because of its localization, it is an important efflux transporter from the cell into the bloodstream. Compared to *ABCB1*, the structure of *ABCC1* is atypical for the ABC transporters. It has five domains: the first domain has an extra NH2-proximal MSD, which contains 5 TM segments and an extracytosolic NH2 terminus, while the remaining four domains have the typical structure of an ABC transporter [37].

1I7I3I3 ABCC2

MRP2, encoded by the *ABCC2* gene, has the same structure as ABCC1 and is located on chromosome 10q23-24. MRP2 is like MDR1 localized at the apical membrane of the cell. The highest expression of MRP2 is in the villi of the proximal jejunum, but it is also expressed in the liver, lung, colon, bile and placenta. MRP2 is very likely involved in the efflux of xenobiotics i.e. Hg elimination, because there is some evidence that the Hg-GSH-conjugate is transported by this protein [32, 35, 37].

1I8 Candidate genes

The candidate genes include those genes which encode proteins that can be assumed to be involved in Hg uptake, metabolism or detoxification (Table 1). SNPs are the most frequent DNA variation in humans. Non-synonymous coding SNPs have the ability to alter the amino acid sequence of the corresponding protein which can affect its function and interaction. Variation in candidate genes can modify Hg toxicokinetics in human individuals, ranging from regular enzyme activity to low or no enzyme activity in poor metabolizers. Based on this fact, non-synonymous SNPs are assumed to have major impact on the phenotype (reviewed by Gundacker et. al [26]). Detailed information about the selected candidate genes is given in Table 1.

Table1. Detailed information on selected candidate genes

Genes/Polymorphism	Homozygous /common allele(%)	Protein/ Enzyme function	Functional part of proteins/enzymes in Hg metabolism	Effects associated with variant allele	References
GCLC-129 (rs17883901) ***GCLM-877*** (rs41303970)	CC:82% CC: 76%	GCL (enzyme of the GSH system)	direct: GCL catalyze the reaction of GSH synthesis	No effect or increased Hg levels has been found	[39-41]
GSTM1 Deletion	+/?: 45%*	GSTM1(enzyme of the GSH system)	Direct: GSTs catalyze the binding reaction of Hg with GSH. Hg-GSH complex can be easily effluxes from the cell	↑ Hg levels (no enzymatic activity by homozygous deletion)	[41, 42]
GSTT1 Deletion	+/?: 83%*	GSTT1(enzyme of the GSH system)		↑Hg levels in interaction with GSTM1(no enzymatic activity by homozygous deletion	[41]
GSTP1-105 (rs1695) ***GSTP1-114*** (rs1138272)	AA: 48% CC:84%	GSTP1 (enzyme of the GSH system		↑Hg levels (↓enzyme activity) only by gene-gene interaction ↓ Hg levels	[40, 41]
MT1a (rs1164085)	AA:54%	MT (metal homeostasis)	Direct:↑metal binding affinity	No effect of Hg levels observed, generally unexplored	[41, 43]
MT2a (rs10636)	GG: 58%			Changes in Fe and Zn levels, no effect of Hg levels observed, generally unexplored	[41, 44]
MT4a (rs11643815)	GG:78%			↑ Hg levels, generally unexplored	[41]
ABCB1 (rs1045642) ***ABCB1*** (rs2032582) ***ABCB1*** **(**rs1128503)	CC: 22% GG: 29% CC: 34%	MDR1/P-gp (Transporter)	Direct: export pump for all metal-GSH conjugations	Haplotype 1-2: "poor Transporter"↓Pgp expression(in placenta too) ↑mRNA , alteration in subtratesspecificities	[36, 38, 45-47]
ABCC1 (rs246221)	TT: 58%	MRP1 (Transporter)		↓transport rate, alteration in membrane localization	[47, 48]
ABCC2 (rs7171620) ***ABCC2*** (rs3740066) ***ABCC2*** (rs1885301)	CC: 76% CC: 51%	MRP2 (Transporter)		↓transport rate (↓mRNA), alteration in membrane localization	[46-48]

*+/- includes homozygous common allele (+/+) and heterozygous genotype (+/-)[41]

1I9 The aims of the study

The study is based on 100 examined Mother-Child-Pairs in Bratislava, Slovakia. The aims of the study were:

1) to determine Hg exposure of the Mother-Child-Pairs by analyzing Hg concentration in erythrocyte samples of maternal blood and cord blood

2) to investigate the potential relationships between genotypes and phenotypes (Hg exposure) of our study participants.

2I Materials and Methods

For this longitudinal study, 114 women were recruited during the third trimester of gestation at the Ruzinovin clinic in Bratislava. Of the 114 recruited study participants, 14 dropped out because of gestational complications. The inclusion criteria included multiple pregnancies, gestosis, metabolic diseases or thyroid dysfunction, premature birth, hypertonia, diabetes mellitus or illegal drug consumption. The study participants were informed about the aims and length of the study and the expense allowance of 25€. Moreover the study participants had to sign a written informed consent. The ethics committee of the University clinic in Bratislava permitted this longitudinal study. Table 2 gives an overview of our study group.

2I1Sampling

"During the 36th-38th week of pregnancy each participant donated 3 x 7 ml of blood. In addition, the women completed a questionnaire about health status, diet, amalgam fillings, education, smoking habits, and area of residence (for questionnaires see appendix).

After birth, cord blood samples (1-3 tubes of 7 ml, respectively) were taken. Immediately after sampling, maternal blood and cord blood samples were centrifuged for 10 minutes at 3000 rpm to separate erythrocytes from blood plasma. All samples were stored at -20°C until further treatment.

Two to eight weeks after birth the women completed a second questionnaire about health status of mother and child and birth outcome. The data on gestational length and newborn anthropometry (birth weight, birth length, head circumference) were taken from the medical records." (Plichta 2013:15 [49])

Table 2. Profile of the study group

		N	Mean ±SD	Range	N (%)
Women	Age [years]	100	31±-5	18-43	
	Hight [cm]	100	167.3±5.5	152-181	
	BMI	100	22.4±3.5	16.0-32.0	
	Pregnancy BMI	98	24.3±3.7	17.7-33.2	
	Parity	100	1.9±1	1-7	
	Gestation length [days]	100	280±7	259-294	
	Amalgam fillings	91	7.8±4.2	1 - 16	
	Fish consumption [g/w]	100	149±176	0-1000	
	Fresh water fish consumption [g/w]	100	19.45±52.8	0-300	
	Marine water fish consumption [g/w]	100	129.3±150	0-800	
	Ery-Hg [µg/kg]	100	1.75±0.8	0.5-4.6	
	Non-smokers				51 (52)
	Current smokers				4 (4)
	Ex-Smoker				43 (44)
Children	Birth weight [g]	100	3441±440	2370-4690	
	Birth length [cm]	100	50.5±1.8	46-55	
	Head circumference [cm]	77	34.4±1.6	30-38	
	Ery-Hg [µg/kg]	100	2.3±1	1.0-6.9	
	Females				43 (43)
	Males				57 (57)

2I2 Preparation and acid digestion of samples

"Prior to use all instruments and sample tubes were cleaned with HNO_3 (Merck, Germany; p.a) mixed with millipore water (dH_20) in a ratio of 1:10. 1.0-1.5 g of each thawed erythrocyte sample was digested with a mixture of 4 mL 69 vol% HNO_3 (Roth, Germany; Supra quality) and 0.75-1.0 mL 30% H_2O_2 (Merck, Germany, p.a.) in a microwave oven [...] [(Table 3)].

We used field blanks (4 ml HNO_3 plus 1 ml H_2O_2) and standard reference material (Seronorm, Trace Elements Whole Blood L-2, 210205) to control measurement quality.

***Table 3**. Program for digestion of blood samples in microwave mls 1200 mega*

Step	*Time*	*Watt*
1	*10:00*	*300*
2	*4:00*	*450*
3	*3:00*	*550*
4	*7:30*	*700*
5	*9:30*	*500*
Ventilation	*3:00*	

After cooling, vessels were rinsed with 2 x 2 ml dH_20. Sample solutions were filled up with 2 ml HCl (Roth, Germany) and dH_20 to a volume of 10 mL. A sample solution aliquot of 4 ml was then decanted in a mercur© tube and volumetrically filled up with dH_20 to 20 ml, respectively.“ (Plichta 2013: 16-17 [49]

2I3 Analysis of Hg

“The samples were analyzed by cold vapor atom fluorescence spectroscopy (CV-AFS) (mercur plus, Analytik Jena, Germany). (Fig.5) One characteristic of Hg^0 is that it is a gas at room temperature. This is the reason why the cold vapor technique is a common method to detect total Hg content. CV-AFS has the advantage of being able to detect even very low concentrations. The method of AFS is based on the optical emission from gas-phase atoms accelerated to higher energy levels. The atom fluorescence then reradiates the absorbed energy. This fluorescent signal enters a quartz window which abuts on a 250 mm long absorption cuvette and goes through a second quartz window directly to the photomultiplier. (Fig.6 [...] [50, 51]*).*

Figure 5. *mercur plus, Analytik Jena [4]*

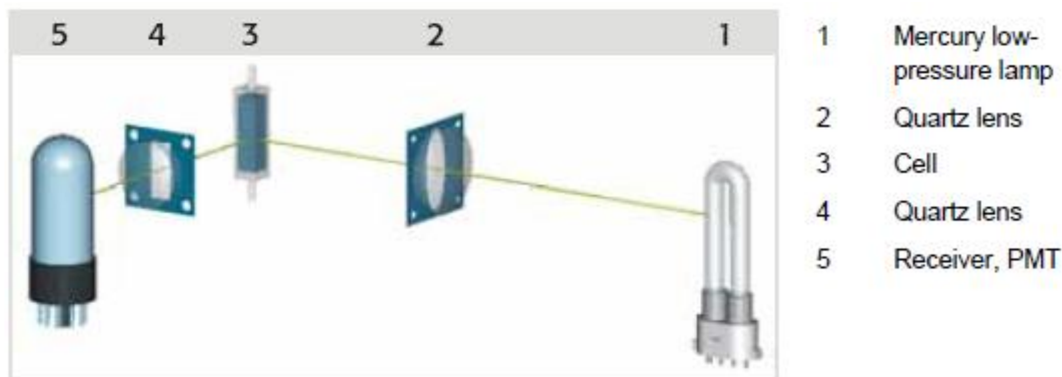
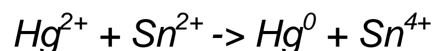


Figure 6. *Schematic diagram of Hg analysis by AFS [4]*

Before the samples can be measured by AFS, the sample solution is transported to the reactor vessels, where the solution is mixed with the reducing solution $SnCl_2$ [20g $SnCl_2$ (Roth, Germany) + 100 ml HCl (Roth, Germany) in 1l $dH_2 0$], which leads to the following reaction:



$$Hg^{2+} + Sn^{2+} \rightarrow Hg^0 + Sn^{4+}$$

On the way to the gas-liquid separator, Argon (gas) is added to the solution and carries the Hg^0 gas atoms to the AFS detector. After each measurement the system is cleaned by HCl. [4, 52]

Hg levels of the reference material (LOT 1003129; 16.1±1.3 µg/L; n=20 and LOT 1003192: 15.2±0.9 µg/L; n=8) lay well within the certified ranges (16.0± 3.2 µg/L and 15.2±0.8 µg/L). The limit of detection (LOD), which is defined as the concentration equivalent to the threefold standard deviation of the blank solution, was 0.16 µg/L.

The Hg contents were measured in duplicate (RSD<10%) by the working curve method.” (Plichta 2013:17-18 [49])

2I3 Genetic analyses

The genetic analyses were conducted in the medgene laboratory, Bratislava, Slovakia. The genomic DNA was extracted out of 0.2 mL maternal blood and 0.2 mL of cord blood by using a NucleoSpin® Blood DNA purification kit (MacheryNagel). The obtained DNA samples were diluted to 30 ng/µL. For detecting the SNPs, the Taqman Method was used. This method is based on the real time polymerase chain reaction (qRT-PCR) which concedes a rapid identification of genes. The qRT-PCR was performed using reagents, Taqman® probes and commercial assays of Applied Biosystems, according to the standard protocol, which means using 60°C annealing temperature and using StepOne™ Real-Time PCR Systems. Furthermore, the Fam and Hex Taqman® probes and primers were pooled in a single 10 µL of reaction volume including 5 µL 2x ABsolute QPCR ROX Mix (Thermo scientific) and 30 ng of genomic DNA. Positive controls for each genotype and one negative control (with water) were tested in every plate (48well). The data was evaluated by StepOne™ softvare Version 2 (Applied Biosystems). Table 4 shows the protocol of the analyzed genotypes. The profile for the standard qRT-PCR included four steps:

1) Pre- PCR read stage at 60°C for 30 seconds (Pre-PCR measurement step);
2) Holding stage at 95°C for 10 minutes (denaturationstep);
3) Cycling stage (40x) Step 1: 95°C 15 sec, Step 2 60°C 1min, the measurement was made at each cycle during step 2;
4) Post PCR read stage 60°C for 30 sec, the post PCR measurements were in this step.

Table 4. PCR protocol of *MT*, *GSTP1*, *GCLC*, *GCLM*, *ABCB1* and *ABCC2* genotypes analysis

Investigated SNP [a]	PCR protocol	
MT1a (rs116408) *GCLC-129* (rs17883901) *GSTP1-105* (rs1695) *GSTP1-114* (rs1138272)	2xABsolute QPCR ROX Mix (Thermo scientific) Forward and reverse primer(10uM) Taqman® probe Fam (Applied Biosystems) Taqman® probe Hex (Applied Biosystems)	5 μL 0.5 μL (each primer) 0.1 μL 0.3 μL
GSTT1 (deletion) *GSTM1* (deletion)	2xABsolute QPCR ROX Mix (Thermo scientific) Forward and reverse primer (10uM) Taqman® probe Hex (Applied Biosystems) (B2M) Taqman® probe Fam (Applied Biosystems) (GSTT1/GSTM1)	5 μL 0.6 μL (each primer) 0.3 μL 0.625 μL
MT2a (rs10636) *ABCB1* (rs1045642) *ABCB1* (rs1128503) *ABCB1* (rs2032582) *ABCC2* (rs3740066) *ABCC2* (rs717620)	2xABsolute QPCR ROX Mix (Thermo scientific) Probe mix (20x) (Applied Biosystems)	5 μL 0.4 μL

a) Reaction volume of 10 μL was used and 30 ng of genomic DNA per reaction was added.

The SNPs *MT4a* (rs41303970), *GCLM-877* (rs41303970) and *ABCC1* (rs246221) were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). 10 μL reaction volume including 2x ABsolute PCR Master Mix (Thermo scientific), 0.5 μL of each primer (10μM) and 30 ng genomic DNA was used for the PCR-RFLP. The condition of the PCR amplification was:

1. Start during the denaturation phase at 95°C for 15 min;
2. 40 cycles at 95°C for 30 seconds;
3. Annealing at 55°C for 30 seconds;
4. Extension at 72°C for 30 seconds;
5. Final extension phase at 72°C for 10 minutes.

After the PCR, an aliquot of the PCR product was digested with 1U of restriction enzymes for 16 hours by recommended temperature. With the help of ethidium bromide, the digestion products were visualized after electrophoresis in 9% PAGE

gels by a constant voltage of 150 V for 2.5 hours in one TBE buffer (Fa. ROTH). Information about primers and digestion conditions are listed in Table 5. To confirm the genotyping results by qRT-PCR or PCR-RFLP, all investigated SNPs were regenotyped in 5% of the samples by using an ABI 3100 automated sequencer (Applied Biosystems).

Table 5. Primer and digestion protocol of MT, GCLM and ABCC1 genotypes PCR-RFLP analysis

Investigated SNP	Primers 5'→ 3'	Restriction enzyme and digestion conditions	Digestion protocol
MT4a (rs11643815)	Fwd: GGATTGAAACCTCATGCACAC Rev: TGTCTGAGCCTCCTTTGCAGATGGA	*HinfI* (Metabion) digest: 37°C 16h, heat inactivation 80°C 20 min	30 µL reaction volume: 10xbuffer B3 (Metabion) 100xBSA 1U *HinfI* (Metabion) 8 µl PCR product
GCLM -877 (rs41303970)	Fwd: GAGACGTGTAGGAAGCCCACC Rev: CGAGAAAGTGCTTCGTAACCG	*HpaII/BsiSI* (Metabion) digest: 55°C 16h, no inactivation	30 µL reaction volume: 10xbuffer BsiSI (incl. BSA) 1U HpaII/BsiSI (Metabion) 8 µL PCR product
ABCC1 (rs246221)	Fwd: GAGCTTAAGGAYCTTGYCTGAA Rev:CGGCTGGGCAGGATCCTTGGAGGAGTACT	*TaqI* (Metabion) digest: 65°C 16h, heat inactivation 80°C 20 min	20µL reaction volume: 10x buffer TaqI (Metabion) 100xBSA (Metabion) 1U TaqI (Metabion) 3µL PCR product

2I4 Statistical analyses

Hg levels were not normally distributed (Liliefors test $p<0.05$). Subsequently, non-parametric tests were used for statistic evaluations. Spearman rank correlation was applied for correlation analysis between lifestyle parameters (diet, education and smoking habits) and Ery-Hg levels. The Mann Whitney U Test (two groups unpaired variables) and the Kruskal Wallis test (three or more groups unpaired variables) were used for group comparison. For analyzing the possible interrelationship between fish consumption and dental amalgam fillings, or between fish consumption and child sex, the Chi-Square test was used. For statistical analyses, metric variables were coded into categorical variables (Table 6). In accordance to allelic frequencies, the genotypes -given as homozygous common allele (wildtype), heterozygous genotype and homozygous variant allele- were combined into two groups, once into homozygous wildtype vs heterozygous and homozygous variants, and second into homozygous variants vs heterozygous and homozygous wildtype. For dual genotype combinations (*GSTP1-105* and *GSTP1-114*) and triple genotype combinations (*ABCB1* rs1045642, rs1128503, rs2032582), the genotypes were grouped into three categories i.e., homozygous wildtype, homozygous variants, and remaining genotypes.

Table 6. Categorization of metric variables

No. of Amalgam fillings	1-5 (n=26)	6-10 (n=39)	11-16 (n=26)	
Fish consumption [g/w]	0 (n=21)	10-100(n=27)	101-250 (n=35)	251-1000 (n=17)
Education level	low[a] (n=27)	middle[b] (n=21)	high[c] (n=52)	
Birth length [cm]	46-49 (n=24)	50-51 (n=46)	52-55 (n=30)	
Birth weight [g]	2370-3000 (n=16)	3010-3500 (n=40)	3530-4000 (n=34)	4030-4690 (n=10)

a: elementary and second modern school; b: apprenticeship, grammar and vocational school; c: college, university

Categorical regression analysis (CATREG) was used to verify the independent effects of exposure modulators (genetic and non-genetic factors) on Ery-Hg level. Variables significantly associated ($p<0.1$) with Ery-Hg levels in the bivariate analysis, were included into CATREG models. The design of each CATREG model is given in Table 7 and 8. The Pratt-coefficient of relative importance was used as criterion for elimination. The non–significant variables were eliminated from the respective model in a stepwise manner ($p>0.05$ and Pratt coefficient <0.05). For statistical calculation SPSS 19.0 was used (SPSS Inc, Chicago, IL). [16]

Table 7. CATREG models on MatBl-Ery-Hg

Mother	**Model 0 (non genetic variable)**	**Model 1**	**Model 2**
	Amalgam fillings Fish consumption	Amalgam fillings Fish consumption	Amalgam fillings Fish consumption
		ABCB1 (rs1045642) (coded in common allele vs. heterozygous and variant allele)	Combination of *ABCB1* (rs1045642, rs1128503, rs2032582) (categorized in common allele, mixed and variant allele)
		ABCB1 (rs2032582)	

Table 8. CATREG models on CordBl-Ery-Hg

Child	Model 0 (non genetic variable)	Model 1	Model 2
	Amalgam fillings Fish consumption	Fish consumption Child sex	Fish consumption Child sex
	Child sex Education level	*GSTP1-105 (*rs1695) (maternal) (common allele vs. heterozygous and variant allele)	Combination of maternal *GSTP1-105* (rs1659) and *GSTP1-114* (rs1138272) (common allele vs. heterozygous and variant allele)
		ABCB1 (rs1045642; maternal) *ABCB1(* rs1128503; maternal) (common allele and heterozygous vs. variant allele) *ABCB1*(rs2032582; maternal) (common allele and heterozygous vs. variant allele) *ABCC2* (rs3740066) (common allele and heterozygous vs. variant allele)	Combination of maternal ABCB1 (rs1045642, rs1128503, rs2032582) (categorized in common allele, mixed and variant allele)

3I Results

3I1 Hg concentrations in maternal blood and cord blood

The mean MatBl-Ery-Hg levels amount to 1.75±0.8 µg/kg. In comparison to the MatBl-Ery-Hg levels, CordBl-Ery-Hg levels were higher with a mean value of 2.27±1 µg/kg (Table 9). The MatBl-Ery-Hg and CordBl-Ery-Hg levels were significantly correlated ($p<0.001$).

Table 9. Hg concentrations in maternal and cord blood

	N	MIN	MAX	25 Percentile	50 Percentile	75 Percentile
MatBl-Ery-Hg [µg/kg]	100	0.51	4.58	1.23	1.56	2.07
CordBl-Ery-Hg [µg/kg]	100	1.03	6.93	1.6	2.05	2.53

3I2 Determinants of Hg exposure

Table 10 shows the correlations between maternal fish consumption, education, number of dental amalgam fillings and maternal and cord blood Hg levels. Smoking habits, gestational age, maternal age and anthropometry are not significantly associated with MatBl-Ery-Hg levels or CordBl-Ery-Hg levels ($p>0.05$, respectively). The marine water fish consumption of our study participants ranged from 0-800 grams per week [g/w], and from 0-300 g/w for fresh water fish consumption.

Table 10. Correlation of Ery-Hg levels and non-genetic factors

	CordBl-Ery-Hg	**MatBl-Ery-Hg**	**Fish consumption**	**Marine water fish consumption**	**Fresh water fish consumption**	**Education**
CordBl-Ery-Hg		**0.697***	**0.325***	**0.300**	**0.290**	**0.239**
MatBl-Ery-Hg	**0.697***		**0.299**	**0.261**	**0.254**	0.101
Fish consumption	**0.325***	**0.299**		**0.941***	**0.339***	**0.308**
Marin water consumption	**0.300**	**0.261**	**0.941***		0.103	**0.291**
Fresh-water-consumption	**0.290**	**0.254**	**0.339***	0.103		**0.220**
Education	**0.239**	0.101	**0.308**	**0.291**	**0.220**	
Amalgam fillings	-0.037	**0.297**	-0.150	-0.153	0.063	-0.133

Spearman correlation coefficients: *** P< 0.001, ** P <0.01, * P <0.05

We found MatBl-Ery-Hg (p<0.01) and CordBl-Ery-Hg (p<0.01) being associated with fish consumption (Fig. 7.A&B). Furthermore, a relation between maternal education level and CordBl-Ery-Hg (p=0.05) was observed. Subsequently, also a significant (p<0.01) association between maternal education and fish consumption was observed (Fig. 7.C).

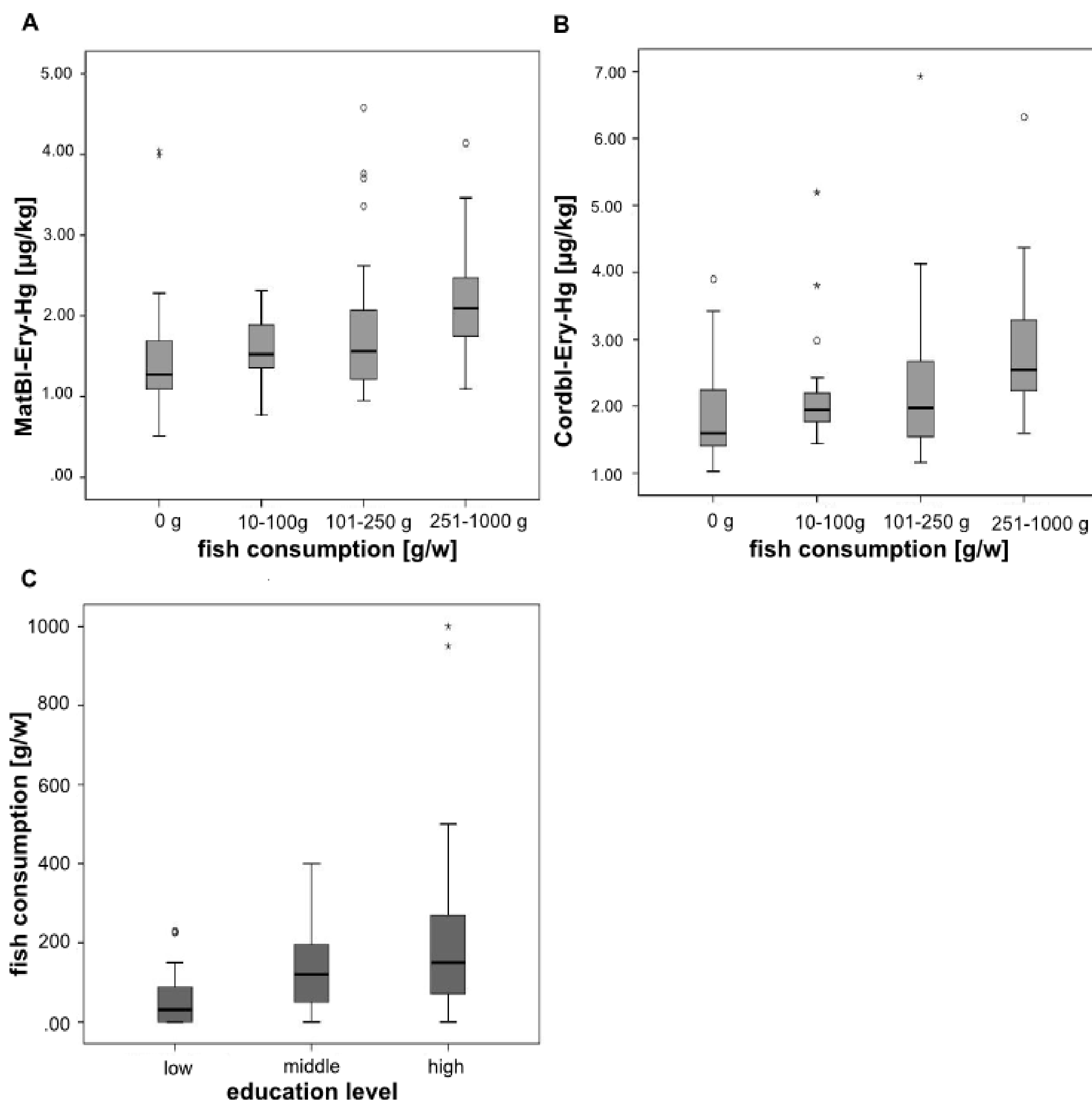
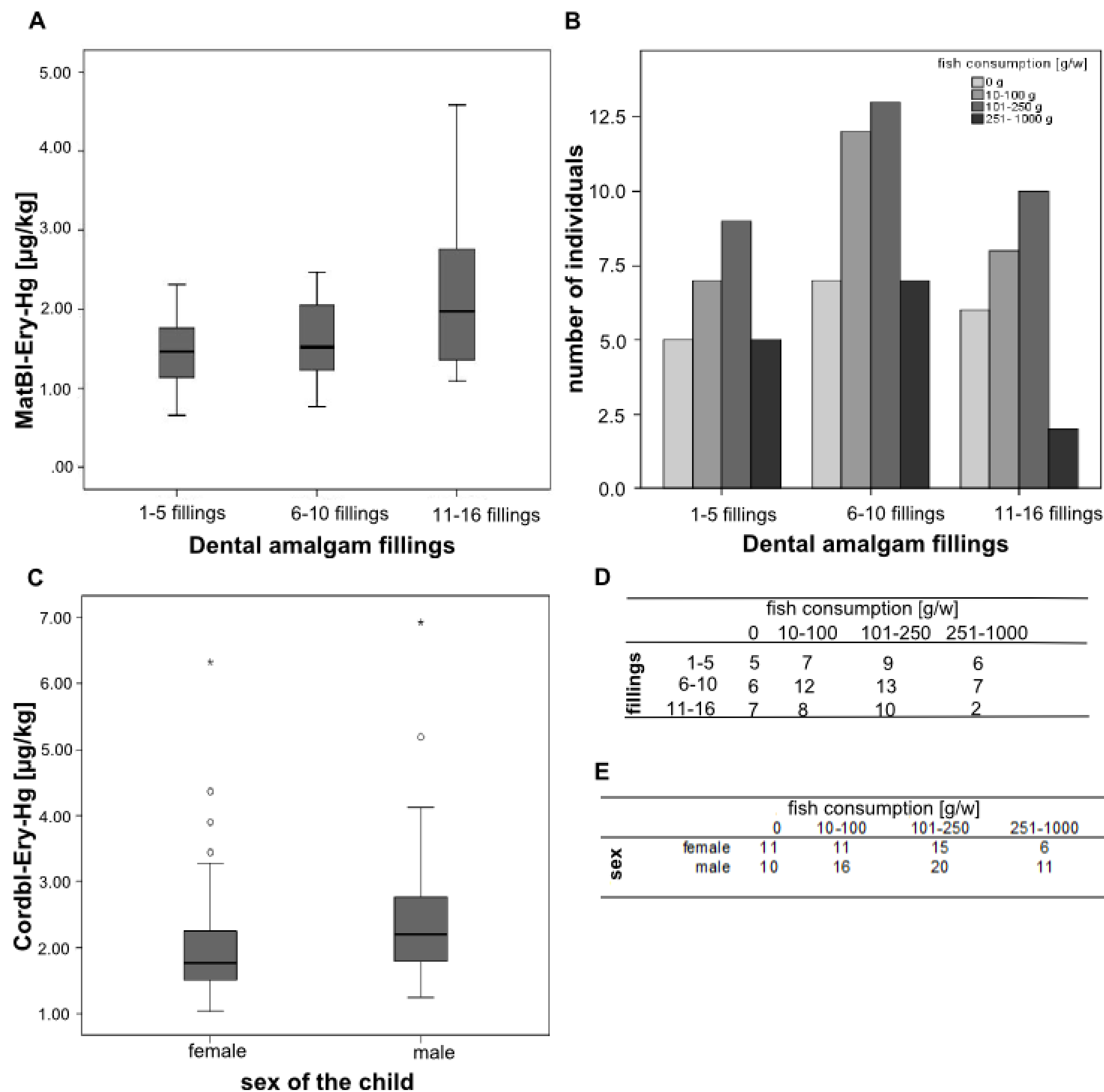


Figure 7. A) MatBl-Ery-Hg levels in relation to fish consumption [0g/w (n=21), 10-100g/w (n=27), 101-250 g/w (n=35), 251-1000 g/w (n=17), (p< 0.05)]. **B**) Association between CordBl-Ery-Hg concentration and fish consumption (p< 0.01). **C)** Total fish consumption in dependence of maternal education [(low= elementary & secondary modern school =27), (middle = apprenticeship, grammar-& vocational school =21), (high=university = 52), (p<0.01)]

Figure 8 (A) illustrates the impact of dental amalgam fillings on the MatBl-Ery-Hg level. A cross tabulation between “dental amalgam fillings” and “fish consumption” was made to prove that the number of dental amalgam fillings and MatBl-Ery-Hg levels are significantly associated and not masked by fish consumption (Fig. 8.D). A p- value of 0.931 confirmed that there was no association between fish consumption

and dental amalgam fillings. Furthermore, it indicates that the child's sex has impact on Hg exposure, because girls (n=43) had significantly lower CordBl-Ery-Hg levels compared to boys (n= 57) (p< 0.01). This result was not influenced by differences in maternal fish consumption (Fig. 8.E, p= 0.748), birth weight (p=0.148) or birth size (p=0.537).



D

fillings	fish consumption [g/w] 0	10-100	101-250	251-1000
1-5	5	7	9	6
6-10	6	12	13	7
11-16	7	8	10	2

E

sex	fish consumption [g/w] 0	10-100	101-250	251-1000
female	11	11	15	6
male	10	16	20	11

Figure. 8 A) MatBl-Ery-Hg levels in relation to dental amalgam fillings [1-5 fillings (n=26), 6-10 fillings (n=39) and 10-16 fillings (n=26) (p<0.01)]. **B)** Fish consumption habits in groups with different numbers of amalgam fillings. **C)** Sex-specific CordBl-Ery-Hg contents [female (n= 43) male (n=57) (p< 0.05)]. **D)** Cross-tabulation of the variables "fish consumption" and "dental amalgam fillings" (Chi²-test p=0.931). **E)** Cross-tabulation of fish consumption and sex of the child (Chi²-test, p=0.748).

3I3 Genetic influence on Hg levels of mother-child-pairs

We tested the genotype frequencies of 100 Caucasian mother-child-pairs (except *GSTM1* and *GSTT1*) to be in Hardy-Weinberg equilibrium by using an online calculator[1]. All genotype frequencies were in Hardy-Weinberg equilibrium, except of *GSTP1-114* (rs1138272) and *GCLM-877* (rs41303970) in mothers and except *ABCB1* (rs1045642) and *ABCB1* (rs2032582) in children. The allele frequencies of the examined SNPs are given in Table 11.

[1] http://www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-2-alleles.html

Table 11. Candidate genes and genotype frequencies

Gene	Genotype	Mother (N)	Child (N)	Frequency in studygroup (%)
MT1a (rs1164085)	AA	51	52	51.5
	CA	40	37	38.5
	CC	9	11	7
MT2a (rs10636)	GG	46	63	54.5
	CG	46	31	38.5
	CC	8	6	7
MT4 (rs11643815)	GG	70	78	74
	GA	27	21	24
	AA	3	1	2
GSTM1	Present	45	50	47.5
	Deleted	55	50	52.5
GSTT1	Present	79	78	78.5
	Deleted	21	22	21.5
GSTP1-105 (rs1695)	AA	58	57	57.5
	GA	35	35	35
	GG	7	8	7.5
GSTP1-114 (rs1138272)	CC	93	85	89
	CT	6	14	10
	TT	1	1	1
GCLC-129 (rs17883901)	CC	83	81	82
	CT	17	19	18
GCLM-877 (rs41303970)	CC	61	59	57.5
	CT	35	40	35
	TT	4	1	7.5
ABCB1 (rs1045642)	GG	21	31	26
	GA	48	40	44
	AA	31	29	30
ABCB1 (rs1128503)	GG	31	36	33.5
	GA	45	45	45
	AA	24	19	21.5
ABCB1 (rs2032582)	CC	31	38	34.5
	CA	45	40	42.5
	AA	24	22	23
ABCC1 (rs246221)	TT	46	53	49.5
	TC	48	37	42.5
	CC	6	10	8
ABCC2 (rs1885301)	GG	17	17	17
	GA	44	46	45
	AA	39	37	38
ABCC2 (rs3740066)	GG	38	37	37.5
	GA	46	47	46.5
	AA	16	16	16
ABCC2 (rs717620)	GG	63	64	63.5
	GA	34	33	33.5
	AA	3	3	3

Two maternal SNPs were significantly associated with MatBl-Ery-Hg levels. First, an *ABCB1* (rs1045642) SNP influenced MatBl-Ery-Hg concentration ($p<0.05$) (Fig. 9). Second, another *ABCB1* Polymorphism (rs2032582) was significantly related to MatBl-Ery-Hg levels ($p< 0.05$, data not shown). A combination of *ABCB1* genotypes (rs104564, rs1128503 and rs2032582) showed that the "GG" genotypes (common allele), was associated with lower MatBl-Ery-Hg concentration compare to the "AA" genotype (variant allele) ($p<0.05$, data not shown).

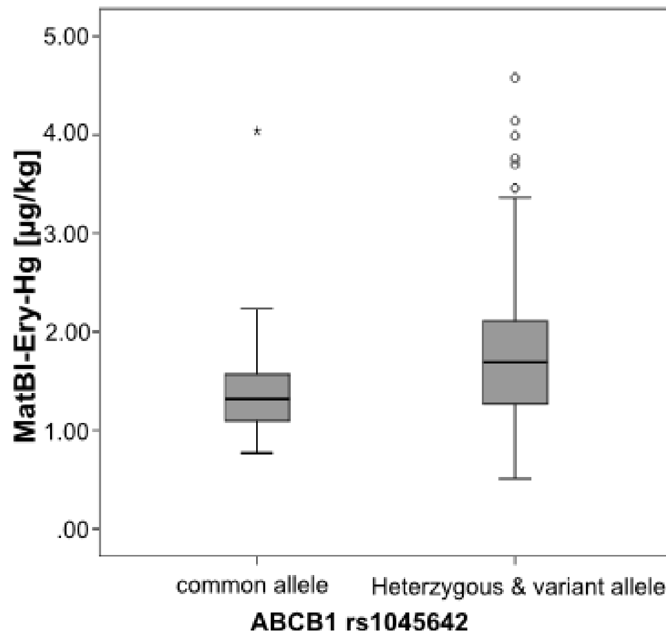


Figure 9. MatBl-Ery-Hg concentration in dependence on *ABCB1* (rs1045642) genotype [common allele (GG, n=21) vs. heterozygous (GA, n=48) and variant allele (AA, n=31) ($p<0.05$)]

With regard to CordBl-Ery-Hg levels, a significant association between variant allele carriers of maternal *ABCB1* genotype (rs1045642) and higher CordBl-Ery-Hg levels was observed ($p<0.05$, data not shown). As shown in Figure 10 (A-C), maternal *ABCB1* genotype (rs 1128503 and rs2032582) and maternal *GSTP1-105* genotype (rs1695) are associated with CordBl-Ery-Hg concentrations. Children carrying a variant of *ABCC2* (rs3740066) show higher Cordbl-Ery Hg levels ($p<0.1$) than children with the common allele (Fig.10.D).

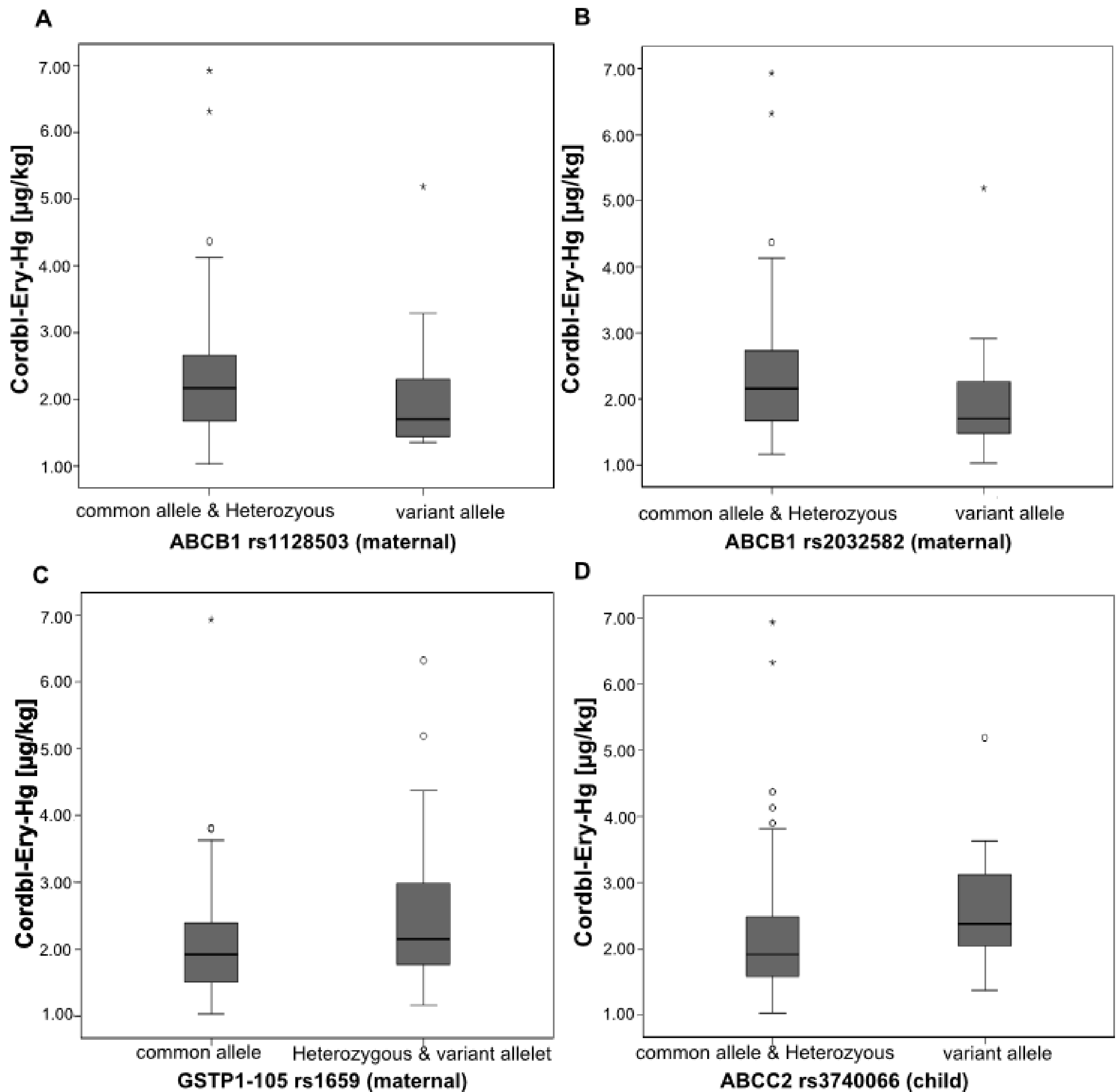


Figure. 10. A) CordBl-Ery-Hg levels in dependence on maternal *ABCB1* (rs1128503) genotype [common allele (GG, n= 31) and heterozygous (GA, n=45) vs. variant allele (AA, n= 24) (p<0.05)]. **B)** CordBl-Ery-Hg concentration in dependence on maternal *ABCB1* (rs203258) genotype [common allele (CC, n=31) and heterozygous (CA, n=45) vs. variant allele (AA, n=24) (p<0.05)]. **C)** CordBl-Ery-Hg levels in dependence on maternal *GSTP1-105* (rs1659) genotype [common allele (AA, n=58) vs. heterozygous (GA, n=35) and variant allele (GG, n=7), (p<0.05)]. **D)** CordBl-Ery-Hg level in dependence on children *ABCC2* genotype (rs3740066) [common allele (GG, n=37) and heterozygous (GA, n=47) vs. variant allele (AA, n=16) (p<0.01)].

A combination of maternal *GSTP1-105* (rs1695) and *GSTP1-114* (rs1138272) genotypes showed that the AA genotype (common allele) is associated with lower CordBl-Ery-Hg levels compared to the GG genotype (variant allele) ($p<0.05$, data not shown). The combination of maternal *ABCB1* genotypes (rs1045642, rs1128503, rs2032582) indicated that homozygous carriers of the common allele (GG) have higher CordBl-Ery-Hg levels than the homozygous variant allele carriers (AA) ($p<0.05$, data not shown).

CATREG analysis of independent predictors of MatBl-Ery-Hg and CordBl-Ery-Hg are given in Table 12 and Table 13, respectively. It was indicated that the independent predictors of MatBl-Ery-Hg concentration include the variables dental amalgam fillings and fish consumption. Furthermore, the *ABCB1* polymorphisms (especially the SNP rs2032582) remain as an independent predictor of MatBl-Ery-Hg levels. CATREG analysis of independent predictors for CordBl-Ery-Hg levels suggested that the *ABCB1* polymorphisms also play an important role in Hg body burden of newborns. Besides *ABCB1*, also *GSTP1-105* (rs1695; maternal) and *ABCC2* genotype (rs3740066; child) explain part of the variation in CordBl-Ery-Hg concentrations. The analysis also indicated that fish consumption during pregnancy, as well as child sex, are independent predictors of Hg exposure.

Table 12. Independent determinants of MatBl-Ery-Hg (CATREG analysis)

Exposure marker	**Design**	**Factor [model]**	**β± S.E**	**P**	**Partial r [R²]**	**Importance coeff. (rank)**
Hg-Ery [µg/kg]	Model 0	Amalgam fillings	0.382±0.101	<0.001	0.396	0.514 (1)
		Fish consumption	0.373±0.094	<0.001	0.388	0.486 (2)
	Model 1	Amalgam fillings	0.357±0.099	0.001	0.379	0.418 (1)
		Fish consumption	0.319±0.096	0.001	0.333	0.365 (2)
		ABCB1 (rs2032582)[a]	0.195±0.086	<0.01	0.214	0.217 (3)
	Model 2	Amalgam fillings	0.363±0.105	0.001	0.383	0.440(1)
		Fish consumption	0.320±0.103	<0.01	0.331	0.374 (2)
		ABCB1 combination[b]	0.172±0.089	<0.05	0.188	0.186 (3)

a) coded as common allele (homozygous)=1, heterozygous= 2 and variant allele (homozygous)=3
b) combination of *ABCB1* genotype (rs1045642, rs1128503, rs2032582) coded into common allele (homozygous)=1, mixed genotypes=2 and variant allele (homozygous)=3

Table 13. Independent determinants of CordBl-Ery-Hg (CATREG analysis)

Exposure marker	Design	Factor [model]	β± S.E	P	Partial r [R²]	Importance coeff.(rank)
Hg-Ery [µg/kg]	Model 0	Fish consumption	0.268±0.097	<0.01	0.276	0.548 (1)
		Child sex	0.242±0.115	<0.05	0.251	0.452 (2)
	Model 1	*ABCB1* (rs1045642) (mother) [a]	0.243±0.087	0.001	0.262	0.299 (2)
		Child sex	0.251±0.123	<0.05	0.269	0.286 (3)
		*ABCB1(*rs1128503) (mother) [b]	0.152±0.088	<0.1	0.149	0.147 (4)
		GSTP1-105 (rs1695) (mother)[c]	0.195±0.102	<0.1	0.193	0.147 (5)
		*ABCC2 (*rs3740066) (child)[b]	0.150±0.081	<0.1	0.167	0.120 (6)
	Model 2	Child sex	0.248±0.115	<0.05	0.218	0.364 (1)
		Fish consumption	0.212±0.110	0.05	0.203	0.340 (2)
		ABCB1 combination (mother)[d]	0.203±0.092	0.01	0.210	0.296 (3)

a) coded as common allele (homozygous) =1, heterozygous=2, variant allele (homozygous)=3
b) coded as common allele (homozygous) & heterozygous=1, variant allele (homozygous)=2
c) coded as common allele (homozygous)=1, heterozygous& homozygous variant allele=2
d) combination of *ABCB1* genotype (rs 1045642, rs1128503, rs2032582); coded in common allele (homozygous)=1, mixed genotypes=2 and variant allele (homozygous)=3

4I Discussion

The Human Biomonitoring Commission provides two HumanBioMonitoring (HBM1 and HBM2) values in order to allow risk assessment regarding Hg exposure of the general population. The HBM1 value represents an alarm value (i.e., 5 µg/L Hg), below which adverse health effects do not occur. The second HBM value (HBM2) - set to 15 µg/L- is an intervention value. At this and higher Hg blood concentration, adverse health effects may occur and medical intervention to decrease Hg blood concentration is necessary. Hg blood levels in the range between the two HBM values (5-15 µg/L) should be verified by repeated analyses and the source of Hg exposure should be identified and eliminated or at least minimized [53].

Concerning our study group all Hg blood levels were below 5 µg/L; the highest value observed was 4.51 µg/L in cord blood[2]. This indicates that the current Hg body burdens of our study participants is not alarming. The here observed Hg blood levels are in good agreement with previous studies conducted in the Bratislava region [54]. Hg blood levels from different studies are summarized in Table 14. Our study group shows lower Hg exposure compared to study groups in Greenland or Canada, which are populations with a traditionally high consumption of fish and/or sea mammals.

Similar to other studies [17], we observed a higher CordBl-Ery-Hg level compared to MatBl-Ery-Hg, i.e., the mean cord blood:maternal blood ratio is 1.4. This ratio may be explained by the fact that MeHg is able to pass the placenta by amino acid carriers, while the analogous carriers on the fetal side are absent or have reduced activity.

[2] Ery-Hg levels [µg/kg] were calculated into whole blood Hg levels [µg/L] by using the formula $Ery-Hg\ levels\ \times weight\ of\ erythrocytes\ (factor\ 1.09) \times hematocrit\ level$

Table 14. Comparison of selected data on Hg levels in maternal and umbilical cord blood

City /Country	Hg-Maternal blood [median, range; µg/L]	Hg-cord blood [median, range µg/L]	References
Bratislava (Slovakia)	0.6; 0.21-1.65	1.2; 0.64-4.51	This study
Vienna (Austria)	0.7; 0.1-5.2	1.1; 0.2-6.8	[16]
Bratislava (Slovakia)	0.5; 0.13-9.89	0.53; 0.13-6.08	[54]
Sweden	0.73; 0.20-2.0	1.4; 0.30-3.8	[55]
Saudi Arabia	1.9;0-206.4	2.9; 0-26.53	[56]
Korea	3.1; 1.7-5.7	5.2; 3.0-9.0	[57]
Greenland*	12.8±13.6; 1.9-75.6	25.3±32.1;2.4-181	[58]
Canada*	10.4±0.4; 2.6-44.2	18.5± 0.4; 2.8-97.0	[59]

*mean values±standard deviation

4l1 Non-genetic factors related to Hg body burden

The main influencing factor of Hg exposure is fish consumption. Marine water fish consumption was correlated to both MatBl-Ery-Hg and CordBl-Ery-Hg levels. The average participant of our study is consuming 7.7 kg per year, which confirms data provided by the National Oceanic and Atmospheric Administration (NOAA), i.e., an average fish consumption of 8.3 kg per year [60]. According to the NOAA report Slovakia is in the lower range of fish consumption comparing EU member states [61]. Based on the average fish consumption of 19.45± 52.8 g/w, none of our study participants exploited the PTWI (WHO). Our study indicates that participants prefer marine water fish over fresh water fish. Canned tuna was the most consumed fish type.

The level of education influences MatBl-Ery-Hg as well as CordBl-Ery-Hg levels. Experts hypothesize that people with a lower education degree have less ability to obtain and/or to understand information concerning a healthy balanced diet [62] Roos et al. [63] summarized that people with a lower socioeconomic status prefer to eat

traditional cuisine, which, in Slovakia, is rich in meat, bread, sauerkraut and less so in vegetables, fish and fruits (recommended healthy food). In this way maternal education level might have influenced dietary habits, which in turn has an impact on Hg exposure in our study group.

Dental amalgam fillings also have a major impact on the Hg body burden of our study participants, i.e., with increasing number of dental amalgam fillings the Hg blood level also are increasing. According to the WHO [64] dental amalgam fillings cause a daily Hg uptake between 1-27 µg. Induced by chewing, tooth brushing or drinking hot beverages, the majority of dental amalgam holders have an uptake of less than 5 µg Hg/d. The amount of Hg uptake depends on the number of dental amalgam surfaces. Conclusively it seems that dental amalgam fillings are an important source of a permanent low level Hg exposure to humans.

In our study group we observed gender-related Hg body burden. Female babies had lower CordBl-Ery-Hg levels compared to male newborns, although there was no gender-related difference in maternal fish consumption or MatBl-Ery-Hg levels. Thomas et al. [65] concluded that female rats have a faster elimination rate compared to male rats. Upon 98 days of MeHg treatment, female rats excreted 54% of the administered MeHg by feces, which was 51% in male rats. The urinary excretion of MeHg in female rats was 7.5% while male rats excreted only 3.2%. Vahter et al. [66] concluded there is a gender-related difference in retention, disturbance and metabolism of MeHg based on sex-specific variations in kinetics, modes of action and susceptibility to toxic metals. Furthermore, it has been shown that there is limited distribution of MeHg to fatty tissue, which might be an explanation why males accumulate more Hg than females [67]. Nevertheless, these data refer to animal experiments or human adults, our results should therefore be interpreted with caution. Further research is required to confirm this finding.

4I2 Genetic factors related to Hg body burden

We found strong evidence that a certain genetic background modifies Hg body burden, especially variations in the *ABCB1* and the *GSTP1-105* genes.

4I2I1 *GSTP1* and Hg exposure

Based on the amino acid substitution caused by *GSTP1-105* (rs1695) SNP and the resulting structural alteration of the enzyme, cysteine residues are not in their common position, which may lead to a loss of ability to bind Hg. Variant carriers (GG) therefore have a threefold lower substrate binding affinity (reviewed by Goodrich et.al [31]). This is indicated by other studies [31, 40, 41] as well as by our findings, i.e., variant carriers of *GSTP1* (single genotypes or combined genotype) have higher Ery-Hg levels. In an *in vitro* study, impaired enzymatic activity of variant carriers by the joint *GSTP1* genotypes combination has been observed. The reduced enzymatic activity of *GSTP1* may result in lowered amounts of exportable GSH-Hg-conjugates followed by higher Hg accumulation rates in the body [31].

4I2I2 *ABCB1* and Hg exposure

Bivariate, as well as CATREG analyses indicate that all investigated *ABCB1* polymorphisms (rs1045642, rs1128503, rs2032582) influence the Hg metabolism in mothers and children.

At position 3435, *ABCB1* (rs1045642) has an exchange of cytosine to thymine, which has been assumed to alter *ABCB1* expression and therefore P-glycoprotein activity. This silent mutation is located in a non-coding and non-promoting area of *ABCB1* [36]. Tanabe et al.[68] assumed that based on its location it remains unclear if the mutation has a direct influence on gene expression of P-glycoprotein. It is more likely that the SNP is associated with another *ABCB1* SNP, e.g. in a promoter/enhancer or intronic regions, which leads to the altered transporter function. Silent mutations can induce an alteration in mRNA folding, which influences mRNA splicing, processing or translational control and regulation of protein expression.[38] In our study group we observed that carriers of the common *ABCB1* allele (rs 1045642; GG) had lower Ery-Hg levels than variant allele carriers. This perhaps is the result of a higher P-glycoprotein level and transporter activity in carriers of the common allele as has been reported in an in *vivo* study on humans (Hoffmeyer [36]). Similar to this *ABCB1* sequence variation, another *ABCB1* polymorphism (rs1128503) also does not result in an amino acid exchange, because of its wobble position. [69]

Tanabe et. al [68] concluded that the transversion of G2677T/A of *ABCB1* (rs2032582) is a missense mutation, which is located in the intracellular side at the MSD2 area of the P-glycoprotein. Based on this transversion, alanine on position 2677 is replaced by the amino acid tyrosine or serine (the latter is the commonest amino acid exchange [70]), which leads to a change from a lipophilic to a hydrophilic residue. Therefore, the amino acid substitution may affect the geometric accuracy of the interaction site and the secondary structure of P-glycoprotein.

Kimchi-Sarfaty et. al [38] observed a linkage between *ABCB1* (rs2032582; G2677A/T), *ABCB1* (rs1045642; C3435T) and *ABCB1* (rs1128503; C1236T), confirming that these SNPs are part of a common haplotype. Our findings on allele frequencies of two *ABCB1* genotypes (rs2032582, rs1128503) support these findings, indicating that the SNPs are a part of a haplotype.

Compared to the currently availabe literature, our results are conflicting with regard to the *ABCB1* (rs1044642) genotype. In Table 15 the current findings on the functional impact of *ABCB1* polymorphisms are summarized.

Table 15. Current data on the functional impact of *ABCB1* polymorphisms

	mRNA expression	**Protein expression**	**Transport function (Substrate)**
ABCB1 (rs1045642)	↓mRNA expression in TT carriers [71]	GG allele: ↑P-gp-expression levels[36]	↑digoxin uptake in TT/TA-carriers[36] ↑fexodenadine levels in CC-carriers [45] ↑loperamide plasma concentration in TT carriers[72]
ABCB1 (rs1128503)	No altered mRNA expression [45]	No data available	No data available
ABCB1 (rs2032582)	↑mRNA expression levels in TT/TA carriers[46]	↓ P-gp-expression in TT/TA carriers[73]	↓digoxin intracellular concentration in TT carriers[69] ↑vincristine efflux in TT carriers[74] ↑fexofenadine levels in CC carriers [45]
ABCB1 (rs1045642, rs2032582)	Variant carriers: ↑expression levels[46]	Variant carriers: ↑protein expression levels [46]	Common carriers: ↑loperamide plasma concentration [72] ↑ amlopidine plasma concentration [75]
ABCB1 (rs1045642, rs1128503, rs2032582)	Variant carriers: ↑expression levels [38]	No data available	Variant carriers: ↑Trypsin degradation [38]

4I2I3 ABCC2 and Hg exposure

ABCC2 (rs3740066) was the only *ABCC2* polymorphism that is statistically associated with CordBl-Ery-Hg levels. Furthermore, it is the only child genotype that is related to CordBl-Ery-Hg levels.

ABCC2 (rs3740066) is a synonymous mutation at exon 28 and shows an exchange of cytosine to thymine at position 3972 [46]. In an *in vivo* study, Benz-de Bretagne et al. [76] observed that study participants, who are carriers of *ABCC2* (rs3740066) variant alleles (TT) have a higher urinary coproporphyrin (UCP I/I+III) ratio, which is indicating decreased MRP2 function. An *in vitro* study carried out by Laechelt et al [77] showed that this variant leads to decreased *ABCC2* protein expression followed by decreased transport function. Both studies confirm our results, that the variant allele carriers (TT) of *ABCC2* (rs3740066) have higher Ery-Hg levels compared to the common allele (CC) carriers, which is perhaps induced by an impaired *ABCC2* protein expression and reduced capability to excrete Hg.

Based on the fact that this *ABCC2* polymorphism (rs3740066) is a silent mutation, a linkage disequilibrium between this *ABCC2* and another *ABCC2* mutation (rs717620) in the promoter region [78] was indicated in several recent studies [46, 77, 79, 80].

5I Conclusion

Due to the fact that none of our study participants exceeded the HBM1 level, the current Hg exposure levels are not of concern. Despite the small amount of fish consumption, we found a strong association between fish consumption and Ery-Hg levels. Another influencing factor of MatBl-Ery-Hg is dental amalgam. Furthermore, we found evidence that genetic variations influence Ery-Hg levels. We observed that the *ABCB1* transporter modulates Hg body burdens. We could demonstrate that carriers of the common *ABCB1* allele have higher Ery-Hg levels compared to variant allele carriers. Also one *ABCC2* SNP (rs3740066) has the potential to modulate CordBl-Ery-Hg levels. Furthermore, we found evidence that carriers of the common *GSTP1-105* allele show lower Hg body burden compared to the variant genotype. In summary, besides fish consumption, genetic variation also is an important influencing factor of prenatal Hg exposure.

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7I Appendix

Questionnaires:

Projekt UM-MUKI: Umweltschadstoffe in Mutter-Kind-Paaren – Belastungssituation im Raum Bratislava-Wien

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Fragebogen 1 (~SSW21)

Datum:………………………………… **ID**…..................

(1) Allgemeines

Name:... Geburtsdatum: _ _ _ _ _ _ _ _

Geburtsort/-land: .... Wohnort: ....

Größe (cm): .... Gewicht (kg):...

Gewicht vor SS (kg):……………………………… SSW:………………………………………

Wievielte SS…………………….. Anzahl Fehlgeburt/en:…………………….

(2) Höchste abgeschlossene Ausbildung, Beruf

☐ Volksschule ☐ Hauptschule ☐ Lehrabschluss ☐ AHS/BHS ☐ Hochschule

☐ andere:……………………………………………………………………………….

Beruf (auch frühere berufliche Tätigkeiten): ....

....

Beruf Partner: ....

(3) Arbeitsplatz

Sind Sie ☐ Studentin bzw. in Ausbildung ☐ berufstätig ☐ nicht berufstätig ☐ Hausfrau

Dauer Berufstätigkeit:☐ >10 Jahre ☐ >5 Jahre ☐ <5 Jahre

Letzte Berufstätigkeit vor …………..Jahren

Frühere Berufstätigkeit(en) ………………………………………………………………….

Mutterschutz seit ☐ gesetzl. Frist ☐ davor………. ……….☐ später……………………..

Branche

☐ Lebensmittel ☐ Textil ☐ Chemie ☐ Kunststoff ☐ Holz ☐ Medizin

☐ Pharma ☐ Kosmetik ☐ Drogerie ☐ Baustoff ☐ Möbel ☐ Tiermedizin

☐ Tierzucht ☐ Sonstiges: …………………

Fragebogen 2 (~SSW 36)

Datum:………………………………….

ID…...................

(1) Allgemeine Daten

Name:.. Wohnort: ....

Gewicht (kg):... SSW:………………………………………….

Verlauf SS: ……………………………………………………………………………………..

(2) Haben Sie Ihren Wohnort geändert?

☐ nein → **(3)**

☐ ja: Wie viele Personen leben in ihrem Haushalt? Anz. Erwachsene: _ _ Anz. Kinder: _ _

Wie lange wohnen Sie schon an Ihrem derzeitigen Wohnort? _ _ Jahre

☐ Einfamilienhaus: ☐ erbaut vor 1945 ☐ erbaut nach 1945

☐ Wohnung: ☐ erbaut vor 1945 ☐ erbaut nach 1945

☐ dicht verbautes Gebiet ☐ Stadtrand ☐ Land

Abstand zu stark befahrener Straße: □ < 50 m □ 50 – 300 m □ > 300 m

Nähe zu Industrie/Gewerbebetrieben: □ <50 m □ 50-300m □ keine Betriebe in der Nähe

□ grundlegende Sanierungsarbeiten innerhalb der letzten 5 Jahre ……………. □ keine

Bodenbelag: □ neu innerhalb der letzen 5 Jahre □ kein neuer Bodenbelag

überwiegender Bodenbelag: □ Teppich □ Laminat □ Kunststoff Holz □ Keramik □ Linoleum

Polstermöbel: □ >10 Jahre alt, □ >5 Jahre alt □ neue Polstermöbel

□ Ledermöbel

□ Verwenden Sie regelmäßig einen Wäschetrockner in der Wohnung? □ nein □ ja

(3) Anamnese und Blutbild

Aktuelle Erkrankungen: □ nein □ ja, welche:……………………………………………

Medikamente: □ nein □ ja, welche:……………………………………………

(4) Haarbehandlungen

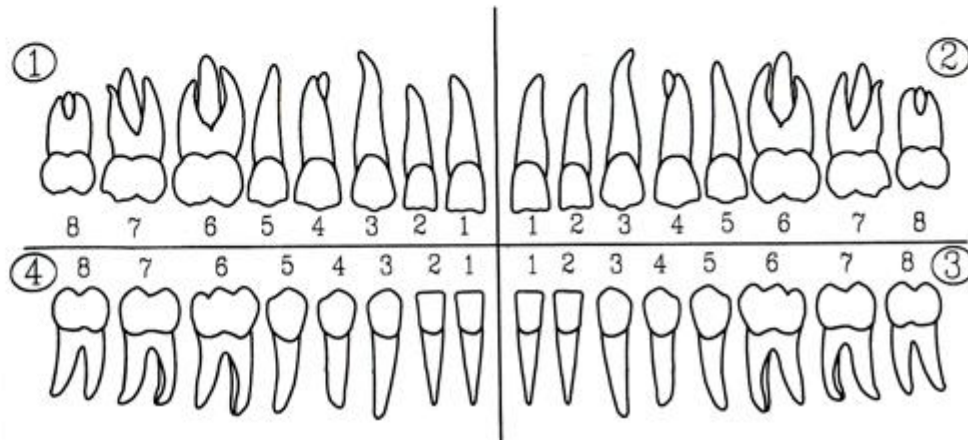
Verwenden Sie Haarfärbemittel? nein ja, welche: ....

Dauerwelle o.ä.: □ nein ja

Wann haben Sie Ihre Haare das letzte Mal gefärbt? vor .... Woche(n)

(5) Zahnstatus

Besitzen Sie Amalgamplomben? nein □ ja, Anzahl Füllungen: ....



Wann war letzte Plombierung? <3 Monate ☐ <6 Monate <9 Monate <1 Jahr

Wurden in der letzten Zeit Amalgam-Plomben entfernt?

nein ja, vor <3 Monaten ☐ <6 Monaten, <9 Monaten, <1 Jahr <2 Jahren

Besitzen Sie Kunststoffplomben /Inlays? nein ja, Anzahl Füllungen: ....

Wann war letzte Plombierung? <3 Monate ☐ <6 Monate <9 Monate <1 Jahr

Wurden in der letzten Zeit Kunststoff-Plomben entfernt?

nein ja, vor <3 Monaten ☐ <6 Monaten, <9 Monaten, <1 Jahr <2 Jahren

(6) Ernährung

Wie oft stehen diese Lebensmittel wöchentlich auf ihrem Speiseplan? Bitte Durchschnittswert für die vergangenen 9 Monate angeben

Fleisch (1 Portion: ca. 150g)

Kalbfleisch/Rindfleisch ☐ nie ☐ 1-2mal ☐ 3-7mal
>7mal

Schweinefleisch ☐ nie ☐ 1-2mal ☐ 3-7mal
>7mal

Geflügel ☐ nie ☐ 1-2mal ☐ 3-7mal
>7mal

Wild nie ☐ 1-2mal ☐ 3-7mal
>7mal

Innereien ☐ nie ☐ 1-2mal ☐ 3-7mal
>7mal

Pilze

Champignons ☐ nie ☐ 1-2mal ☐ 3-7mal ☐
>7mal

Andere Pilze ☐ nie ☐ 1-2mal ☐ 3-7mal
>7mal

Getränke

Leitungswasser (hier: <u>tägliche</u> Aufnahme) ☐ <1/2 L ☐ <1L ☐ <2L >2L

Mineralwasser PET ☐ nie ☐ …………L/Woche

Mineralwasser Glas ☐ nie ☐ …………L/Woche

Softdrinks PET-Flaschen ☐ nie ☐ …………L/Woche

Softdrinks Tetrapak ☐ nie ☐ …………L/Woche

Fruchtsaft Tetrapak ☐ nie ☐ …………L/Woche

Glas Milch ☐ nie ☐ 1-2mal ☐ 3-7mal
>7mal

Tasse Kaffee nie ☐ 1-2mal ☐ 3-7mal
>7mal

Tasse Schwarztee nie ☐ 1-2mal ☐ 3-7mal
>7mal

Tasse Grüntee nie ☐ 1-2mal ☐ 3-7mal
>7mal

1/8 L Rotwein nie ☐ 1-2mal ☐ 3-7mal
>7mal

1/8 L Weißwein/Champagner	nie	☐ 1-2mal	☐ 3-7mal
>7mal			
0,3 L Bier	nie	☐ 1-2mal	☐ 3-7mal
>7mal			
Stamperl Schnaps (2 cL)	nie	☐ 1-2mal	☐ 3-7mal
>7mal			

Schalentiere (1 Portion: ca. 100g)

Muscheln (Austern,…)	☐ nie	☐ 1-2mal	☐ 3-7mal
>7mal			
Krebse (Garnelen, Shrimps, Krabben)	☐ nie	☐ 1-2mal	☐ 3-7mal
>7mal			
Tintenfisch (Oktopus, Sepia, Calamari)	☐ nie	☐ 1-2mal	☐ 3-7mal
>7mal			

Fisch (1 Portion: 100-150g)

Sardinen, Sardellen	☐ nie	☐ …………g/Woche
Dosen-Thunfisch	☐ nie	☐ …………g/Woche
Thunfischsteak	☐ nie	☐ …………g/Woche
Lachs (geräuchert, Steak)	☐ nie	☐ …………g/Woche
Sushi	☐ nie	☐ …………g/Woche
Hering (Matjes, „Russen“,…)	☐ nie	☐ …………g/Woche
Haifisch (Steak, Schillerlocke)	☐ nie	☐ …………g/Woche
Schwertfischsteak	☐ nie	☐ …………g/Woche
Dorsch/Kabeljau	☐ nie	☐ …………g/Woche
Fischstäbchen	☐ nie	☐ …………g/Woche
Scholle, Seezunge	☐ nie	☐ …………g/Woche

Forelle ☐ nie ☐ ………..g/Woche

Karpfen ☐ nie ☐ ………..g/Woche

Hecht ☐ nie ☐ ………..g/Woche

Zander ☐ nie ☐ ………..g/Woche

……………………………….. ☐ nie ☐ ………..g/Woche

……………………………….. ☐ nie ☐ ………..g/Woche

……………………………….. ☐ nie ☐ ………..g/Woche

Take away food/ Fast Food/Dosennahrung

Hamburger/Ähnliches ☐ nie ☐ 1-2mal ☐ 3-7mal
>7mal

Pommes Frites ☐ nie ☐ 1-2mal ☐ 3-7mal
>7mal

„Take away“ in Papierkartons ☐ nie ☐ 1-2mal ☐ 3-7mal
>7mal

„Take away“- Getränke im Papierbecher ☐ nie ☐ 1-2mal ☐ 3-7mal
>7mal

Mikrowellen-Popcorn ☐ nie ☐ 1-2mal ☐ 3-7mal
>7mal

Lebensmittel aus Konserven? ☐ nie ☐ 1-2mal ☐ 3-7mal
>7mal

<u>(7) Rauchen</u>

Sind Sie

☐ Nichtraucherin

frühere Raucherin: Wie viele Jahre haben Sie insgesamt geraucht? ………. Jahre

Raucherin: Seit wie vielen Jahren rauchen Sie? ……….Jahre

Ø Anzahl an Zigaretten: ……………….pro Tag

Wie viele Personen in Ihrem Haushalt sind Raucher? ……Personen

(8) Arbeitsplatz

Hat sich Ihr Arbeitsplatz verändert? □ nein → **(9)**

□ ja: Sind Sie □ Studentin bzw. in Ausbildung berufstätig nicht berufstätig Hausfrau

Dauer Beruftstätigkeit: >10 Jahre >5 Jahre <5 Jahre

Letzte Beruftstätigkeit vor …………..Jahren

Frühere Berufstätigkeit …………..

Mutterschutz seit gesetzl. Frist davor………. ………. später…………………

Branche

Lebensmittel Textil Chemie Kunststoff Holz Medizin

Pharma Kosmetik Drogerie Baustoff Möbel Tiermedizin

Tierzucht Sonstiges: ………………….

Art des Arbeitsplatzes

Büro Produktion Verkauf: ………………

Labor Reinigung chemische Reinigung Arztpraxis Zahnarztpraxis

Fotografie Friseurin Kosmetikerin Sonstiges: ………………….

Bestand Exposition mit (auch bei Studentinnen und Hausfrauen abfragen!)

Reinigungsmitteln

Chemikalien, wenn bekannt (z.B.: Imprägniermittel, Epoxyklebern,…):……………

Bioziden, wenn bekannt: …………

Wurden in den letzen 5 Jahren grundlegende Sanierungsarbeiten am Arbeitsplatz durchgeführt? nein ja …………….

überwiegender Bodenbelag am Arbeitsplatz:

 Teppich Laminat Kunststoff Holz Keramik Linoleum

Sehr häufige / reichliche Anwendung von Raumpflegeprodukten (Imprägnier-, Polier-, Desinfektionsmitteln etc.) nein ja:…………………..

(9) Freizeit

Verhalten vor Schwangerschaft

Tragen von „Funktions“-Sportbekleidung (antibakteriell/geruchshemmend/wasser o. schmutzabweisend)/Woche nie 1-2mal >3mal

Verwendung von Bastelmaterialien, Bastelklebern

 nie 1-2mal/Monat 1-2mal/Woche

Verhalten während Schwangerschaft

Tragen von Funktions-Sportbekleidung (antibakteriell/geruchshemmend/wasser- o. schmutzabweisend)/Woche nie 1-2mal >3mal

Verwendung von Bastelmaterialien, Bastelklebern

 nie 1-2mal/Monat 1-2mal/Woche

HERZLICHEN DANK FÜR IHRE MITARBEIT!

Projekt UM-MUKI: Umweltschadstoffe in Mutter-Kind-Paaren – Belastungssituation im Raum Bratislava-Wien

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Fragebogen 3 (post partum)

Datum:………………………………….

ID…...................

Name:...

Daten Kind

Geburtsdatum: .... Name: ....

Geschlecht: .... Größe:...

Gewicht: .... Kopfumfang: ....

APGAR: .... Schwangerschaftsdauer: in Wochen: ....

Gesundheitszustand

Mutter:……………………………………………………………………………………………………

Gesundheitszustand

Kind:………………………………………………………………………………………………………

Geburtsverlauf

☐ Spontangeburt ☐ Einleitung ☐ Sectio

Anästhesie: ☐ nein ☐ ja: ☐ Vollnarkose ☐ Epiduralanästhesie

Auffälligkeiten Nabelschnur? ....

Auffälligkeiten Mekonium? ....

Auffälligkeiten Fruchtwasser? ....

Auffälligkeiten Plazenta? ....

Blutbild Kind

Apgar:…………………………….. BE:……………………………… Hb:……………………………………………

Nabelschnur-Blut pH art.:…………………… Nabelschnur-Blut pH ven.:……………………………………..

HERZLICHEN DANK FÜR IHRE MITARBEIT!

8I Curriculum Vitae

Persönliche Daten:

Name: Mag. Veronika Plichta
E-Mail: veronika.plichta@gmail.com

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11/2011-06/2013	Diplomarbeiten für die Studienzweige Anthropologie und Ökologie an der Medizinischen Genetik, Medizinischen Universität Wien
2008-	Diplomstudium Anthropologie (Schwerpunkt Humanökologie) an der Universität Wien
2008-21.8.2013	Diplomstudium Ökologie (Schwerpunkt Humanökologie) an der Universität Wien
2006-2008	Diplomstudium der Biologie an der Universität Wien
2005-2006	Diplomstudium der Molekularen Biologie an der Universität Wien
1997-2005	BG Rahlgasse Wien, Matura am 07.06.2005
1993-1997	VS Notre Dame de Sion, Wien

Berufliche Qualifikation

11/2012 – bis dato	wissenschaftliche Mitarbeit am Projekt: ***„Mercury toxicokinetics in human term placenta: functional proof and localization of involved candidate proteins“***
11/2011 – 11/2012	wissenschaftliche Mitarbeit am Projekt: ***„UM-MUKI: Umweltschadstoffe in Mutter-Kind-Paaren-Belastungssituation“*** Diplomarbeit betreut von Prof. Gundacker, Medizinische Genetik, Medizinische Universität Wien
4/2013,4/2012	Tutorin im Humangenetisches Praktikum für Mediziner (Block 5) Medizinischen Universität Wien
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