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CYP3A genes and the association between prenatal methylmercury exposure and neurodevelopment

Sabrina Llop[a,b], **Van Tran**[c], **Ferran Ballester**[a,b], **Fabio Barbone**[d,e], **Aikaterini Sofianou-Katsoulis**[f], **Jordi Sunyer**[b,g,h,i], **Karin Engström**[j], **Ayman Alhamdow**[k], **Tanzy M Love**[c], **Gene E Watson**[c], **Mariona Bustamante**[b,g,h,l], **Mario Murcia**[a,b], **Carmen Iñiguez**[a,b], **Conrad F Shamlaye**[m], **Valentina Rosolen**[d], **Marika Mariuz**[d], **Milena Horvat**[n], **Janja S Tratnik**[n], **Darja Mazej**[n], **Edwin van Wijngaarden**[c], **Philip W Davidson**[c], **Gary J Myers**[c], **Matthew D Rand**[c], and **Karin Broberg**[k]

[a]Epidemiology and Environmental Health Joint Research Unit, FISABIO Universitat Jaume I Universitat de Vale ncia, Av. Catalunya 21, 46020, V alencia, Spain

[b]Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Av. Monforte de Lemos, 3-5. Pabellón 11, 28029, Madrid, Spain

[c]University of Rochester Medical Center, School of Medicine and Dentistry, 601 Elmwood Ave, Box 671, Rochester, NY 14642, USA

[d]Department of Medical and Biological Sciences, University of Udine, via Colugna 50, 33100 Udine, Italy

[e]Institute for Maternal and Child Health IRCCS “Burlo Garofolo”, via dell'Istria 65/1, 34137 Trieste, Italy

[f]Department of Social and Developmental Paediatrics Institute of Child Health, Athens, Greece

[g]ISGlobal, Centre for Research in Environmental Epidemiology (CREAL), Av. Aiguader 88, 08003, Barcelona, Spain

[h]IMIM (Hospital del Mar Medical Research Institute), Av. Aiguader 88, 08003, Barcelona, Spain

[i]Universitat Pompeu Fabra (UPF), Av. Aiguader 88, 08003, Barcelona, Spain

[j]Division of Occupational and Environmental Medicine, Lund University, 22185 Lund, Sweden

[k]Institute of Environmental Medicine, Karolinska Institutet, Nobels väg 13, 17177 Stockholm, Sweden

[l]Genomics and Disease Group, Bioinformatics and Genomics Program, Centre for Genomic Regulation (CRG), Av. Aiguader 88, 08003, Barcelona, Spain

[m]The Child Development Centre, Ministry of Health, Mahé, Seychelles

Corresponding author Karin Broberg, Institute of Environmental Medicine (IMM), C6, Metals and Health, Box 210, 171 77, Stockholm, Sweden, karin.broberg@ki.se.

[n]Department of Environmental Sciences, Jozef Stefan Institute, Jamova cesta 39, Si-1000, Ljubljana, Slovenia

Abstract

Background—Results on the association between prenatal exposure to methylmercury (MeHg) and child neuropsychological development are heterogeneous. Underlying genetic differences across study populations could contribute to this varied response to MeHg. Studies in *Drosophila* have identified the cytochrome p450 3A (CYP3A) family as candidate MeHg susceptibility genes.

Objectives—We evaluated whether genetic variation in CYP3A genes influences the association between prenatal exposure to MeHg and child neuropsychological development.

Methods—The study population included 2639 children from three birth cohort studies: two subcohorts in Seychelles (SCDS) (n =1160, 20 and 30 months of age, studied during the years 2001–2012), two subcohorts from Spain (INMA) (n=625, 14 months of age, 2003–2009), and two subcohorts from Italy and Greece (PHIME) (n=854, 18 months of age, 2006–2011). Total mercury, as a surrogate of MeHg, was analysed in maternal hair and/or cord blood samples. Neuropsychological development was evaluated using Bayley Scales of Infant Development (BSID). Three functional polymorphisms in the CYP3A family were analysed: rs2257401 (*CYP3A7*), rs776746 (*CYP3A5*), and rs2740574 (*CYP3A4*).

Results—There was no association between CYP3A polymorphisms and cord mercury concentrations. The scores for the BSID mental scale improved with increasing cord blood mercury concentrations for carriers of the most active alleles (β[95%CI]:=2.9[1.53,4.27] for *CYP3A7* rs2257401 GG+GC, 2.51[1.04,3.98] for *CYP3A5* rs776746 AA+AG and 2.31[0.12,4.50] for *CYP3A4* rs2740574 GG+AG). This association was near the null for *CYP3A7* CC, *CYP3A5* GG and *CYP3A4* AA genotypes. The interaction between the CYP3A genes and total mercury was significant ($p<0.05$) in European cohorts only.

Conclusions—Our results suggest that the polymorphisms in CYP3A genes may modify the response to dietary MeHg exposure during early life development.

Keywords

Methylmercury; cognitive; *CYP3A* polymorphisms; neurotoxicity; birth cohort; prenatal exposure

1. Introduction

Methylmercury (MeHg) is a ubiquitous environmental toxicant that derives from both natural sources and human activity (World Health Organization (WHO), 2007a). MeHg is present in almost all aquatic species as a result of the methylation of inorganic mercury by microorganisms present in sediments (Parks et al., 2013) and subsequent bioaccumulation up the food chain. Human exposure occurs almost exclusively through consumption of fish and marine mammals. About 95% of the MeHg ingested from fish is absorbed into the bloodstream and readily crosses the placental and blood–brain barrier where it poses the greatest risk for developmental neurotoxicity (World Health Organization (WHO), 2007b).

Several epidemiologic studies have examined the consequences of prenatal MeHg exposure from maternal consumption of fish or seafood on child cognitive and motor development and have found conflicting results, with some studies reporting adverse associations and others finding no influence of exposure on developmental outcomes (Davidson et al., 2008; Grandjean et al., 1997; Llop et al., 2012; Valent et al., 2013). Methodological differences, co-exposures to several environmental pollutants and nutritional factors may have contributed to this observed heterogeneity in effect estimates. In addition, it has been postulated that individual and population genetic differences may also influence MeHg toxicity (Llop et al., 2015; National Research Council, 2000).

Only a few studies have addressed the role of genetics in MeHg toxicity, with most studies based on adult populations (Llop et al., 2015). Candidate genes in the glutathione (GSH) metabolism pathway have been primarily considered since the formation of MeHg-GSH conjugates are thought to be key to excretion (Ballatori and Clarkson, 1983; Barcelos et al., 2013; Custodio et al., 2004; Engström et al., 2008; Engström et al., 2016; Gundacker et al., 2007). However, results have been inconsistent regarding the modifying role of these genes. In an attempt to identify MeHg tolerance and susceptibility genes through an unbiased transcriptomic screen using developing neural tissue of *Drosophila* several members of the Cytochrome p450 (CYPs) family were resolved as gene candidates (Mahapatra et al., 2010). CYPs are a superfamily of enzymes involved in oxidative metabolism of xenobiotics. Genetic polymorphism in these drug metabolizing enzymes is considered to be a major contributor to individual susceptibility to environmental, chemical and drug toxicity (Johansson and Ingelman-Sundberg, 2011).

Functional studies in Drosophila showed that ectopic expression of the *Drosophila CYP6g1* gene, as well as expression of its human homolog *CYP3A4*, in flies, conferred tolerance to developmental MeHg toxicity (Rand et al., 2012). The human CYP3A subfamily is comprised of four distinct genes: *CYP3A4*, *CYP3A5*, *CYP3A7* and *CYP3A43*, which are located in close proximity on chromosome 7. CYP3A4, CYP3A5, CYP3A7 are predominantly expressed in the liver, kidney and gut tissues where they catalyze drug and xenobiotic metabolism (Anzenbacher and Anzenbacherová, 2001). CYP3A enzymes are also essential for the synthesis of endobiotics, such as sex hormones and fatty acids (Hasler, 1999; Zanger and Schwab, 2013) that are crucial in nervous system development. In a developmentally regulated process CYP3A7 is preferentially expressed in human fetal liver which is replaced with CYP3A4 expression postnatally (Hakkola et al., 1998). The expression and function of CYP3A genes in extra-hepatic tissues are less well characterized, nonetheless there is evidence for CYP3A transcripts in developing brain (https://www.ebi.ac.uk/gxa/home). While a direct role of CYPs in MeHg metabolism remains under investigation (Rand et al., unpublished observations), early studies have shown an ability of liver microsomes to biotransform MeHg to inorganic Hg *in vitro* (Nakayama, 1976). Since MeHg de-methylation is recognized as a rate-limiting step in MeHg elimination (Farris et al., 1993; Smith et al., 1994), and correspondingly dictates the body burden of MeHg, a potential enzymatic role for CYPs in mediating MeHg metabolism presents an attractive hypothesis to explore in population based studies.

Several single nucleotide polymorphisms (SNPs) in humans affect CYP3A expression and activity (Lamba et al., 2002). The *CYP3A4* SNP rs2740574 (also referred to as CYP3A4*1B, by The Human Cytochrome P450 (CYP) Allele Nomenclature Database, http://www.cypalleles.ki.se/) is a change in the promoter region that can potentially give higher expression levels of *CYP3A4* (Lamba et al., 2002). *CYP3A5* rs776746 SNP (CYP3A5*3A) encodes a truncated mRNA transcript, resulting in no protein expression (Knops et al., 2015). *CYP3A7* rs2257401 (CYP3A7*2) is a non-synonymous coding SNP that yields an enzyme with increased catalytic efficiency (Rodriguez-Antona et al., 2005). In this study we examined whether these polymorphic variants of CYP3A genes, which predict different levels of CYP activity, modify the association between prenatal exposure to MeHg and neurodevelomental outcomes in children. We studied three birth cohorts from coastal populations; the Seychelles Child Development Study (SCDS), INMA–Environment and Childhood in Spain, and PHIME (Public Health Impact of long-term, low-level Mixed Element Exposure in susceptible population strata) in Italy and Greece. A lack of adverse neurodevelopmental effects during infancy associated with prenatal MeHg in these birth cohorts has been previously reported (Davidson et al., 2008; Llop et al., 2012; Strain et al., 2015; Valent et al., 2013).

2. Methods

2.1 Study population

The study participants were children from three established birth cohort studies: one from the Republic of Seychelles and two from the Mediterranean region. The study in Seychelles (SCDS) included two subcohorts: Nutrition Cohort 1(NC1) and 2 (NC2). The studies from the Mediterranean area encompassed two subcohorts from Spain (INMA study) and two subcohorts from Italy and Greece (PHIME project). SCDS is a longitudinal observational study conducted in the Republic of Seychelles, an archipelago of 115 islands in the Indian Ocean (Strain et al., 2015); most of the population resides on the island of Mahé where the study was conducted and the population is of mixed African, European and East Asian descent. The overall aim of the SCDS is to investigate the associations between child development outcomes and MeHg and nutrients from maternal fish consumption during pregnancy. Healthy mothers were recruited to NC1 and NC2 during their first antenatal visit (from 14 weeks of gestation) at eight health centres across Mahé. NC1 mothers (n=301) were recruited in 2001 (Davidson et al., 2008) and NC2 mothers (n=1535) were enrolled from 2008 until 2011 (Strain et al., 2015). Inclusion criteria included being native Seychellois, at least 16 years of age, having a singleton pregnancy, and no obvious health concerns. Exclusion criteria included low birth weight and serious perinatal or neurological problems. Neurodevelopment evaluations took place at 30 months of age for NC1 (n=276) and at 20 months of age for NC2 (n=1,457) children.

INMA is a multicentre birth cohort study, which aims to investigate the effect of environmental exposures and diet during pregnancy on fetal and child development in different geographical areas of Spain. Details of the INMA project and sampling procedures have been described elsewhere (Guxens et al., 2012). Briefly, pregnant women in this study were recruited during the 1st trimester of pregnancy (n=1,512) and followed until delivery

(n=1,409) in two regions of Spain: Valencia and Sabadell (2003–2007). The inclusion criteria were: at least 16 years of age, 10–13 weeks of gestation, singleton pregnancy, intention of undergoing follow-up and delivering in the corresponding centre of reference, no impediment for communication, and no chronic disease prior to pregnancy. Neurodevelopment evaluations took place around 14 months of age (n=1,253). The data of these two subcohorts was combined since they shared the same study protocol.

PHIME is a multicentre project that aims to evaluate the health effects of long-term exposure to low levels of metals, such as the effects of MeHg on the nervous system. This study included participants from Italy and Greece (Valent et al., 2013); the study population consisted of 1,366 women recruited in the Northern Adriatic province of Trieste (Italy), and the Greek islands of Lesvos, Chios, Samos, and Leros in the Eastern Aegean Sea. The pregnant women eligible for recruitment were: residents of the study areas for at least 2 years, at least 18 years of age, and no absence from the study area for more than 6 weeks during pregnancy, no history of drug abuse, no serious health problems or complications of pregnancy or delivery, and no twin gestation. At recruitment, eligible women were approached for consent after their routine morphologic ultrasound scan between 20 and 22 gestational weeks (Italy, 2007–2009), or during their hospital stay for delivery (Greece, 2006–2009). Neurodevelopment evaluations took place around 18 months of age (n=632 in Italy and n=350 in Greece). The final study population consisted of children with available information on prenatal mercury exposure, CYP3A genotype of the child, and neurodevelopment test scores: 1160 from SCDS (211 from NC1 and 949 from NC2), 625 from INMA, and 854 from PHIME (573 from Italy and 281 from Greece). Women participating in the original studies signed a written informed consent form in each phase. The Seychelles Ethics Board, the Research Subjects Review Board at the University of Rochester, the Ethics Committees of La Fe Hospital in Valencia, the Institut Municipal d'Assiste ncia Sanitaria in Barcelona, the Burlo Garofolo in Trieste, the Institute of Child Health in Athens, and Lund University in Lund approved the study.

2.2 Mercury exposure

In SCDS NC1, INMA and PHIME, total mercury (THg) concentrations were available in cord whole blood samples. THg was for all cohorts measured by thermal decomposition, amalgamation, and atomic absorption spectrometry. For the SCDS NC1, THg was measured at the University of Rochester (Cernichiari et al., 1995). For INMA, THg was measured in the Public Health Laboratory in Alava (LSPPV), Basque Country (Ramon et al., 2011). For PHIME, THg was measured in the laboratory of the Department of Environmental Sciences at the Jozef Stefan Institute in Ljubljana, Slovenia (Miklavcic et al., 2013). The limit of quantification (LOQ) of the procedure was 1.9 ppb in SCDS, 0.07 ppb in PHIME, and 2.0 ppb in INMA. In INMA there were 30 samples below the LOQ and for those we used the approximation LOQ/√2. An interlaboratory comparison was made using INMA cord blood samples (n=12); where THg concentrations in these samples were analyzed at the Jozef Stefan Institute (PHIME laboratory) and the LSPPV (INMA laboratory). The correlation between the concentrations obtained by the two laboratories was high (Spearman correlation coefficient=0.993, $p<0.001$).

Maternal hair samples were available at delivery to determine prenatal THg exposure among SCDS NC1 and NC2 participants. THg was measured by the standard technique of atomic absorption spectroscopy (Cernichiari et al., 1995) at the University of Rochester in the longest hair segment available to reflect exposure throughout pregnancy. The LOQ was 0.61ppm. THg was also analyzed in maternal hair samples from the PHIME participants collected in Italy between the weeks 20–22 and in Greece at delivery. THg was analyzed in the 1 cm of hair closest to scalp at the Jozef Stefan Institute by thermal combustion, amalgamation, and atomic absorption spectrometry using a direct mercury analyzer (Milestone, USA). The LOQ of the procedure was 0.7 ng/g in PHIME (Miklavcic et al., 2013).

2.3 Genetic analysis

DNA was extracted from cord blood (cord tissue for the Italian samples) by the Qiagen DNA Blood Mini kit (Qiagen, Hilden Germany; for SCDS and PHIME), or by the Chemagen protocol (Baesweiler, Germany; for INMA).

We focused on SNPs with a known functional impact on CYP3A expression and activity. Three SNPs were analysed, one in each of the CYP genes: rs2257401 (*CYP3A7*), rs776746 (*CYP3A5*), and rs2740574 (*CYP3A4*). All SNPs were analyzed by Taqman assays apart for the analysis of rs776746 and rs2740574 in INMA, where we took advantage of a genome-wide genotyping already performed (rs2257401 was not present on the BeadChip).

TaqMan allelic discrimination was performed on an ABI 7900 instrument (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. A random selection (at least 5%) of the samples were re-analyzed for quality control purposes with perfect agreement between original and repeat genotyping runs for all SNPs. Differences in genotyping efficiency account for different sample sizes for each SNP. The identification numbers for each assay designed by Thermofisher Scientific are found in (Supplementary Table S1).

Genotyping of rs776746 and rs2740574 in INMA was performed using the Human Omni1-Quad BeadChip (lllumina, San Diego, CA, USA) at the Spanish National Genotyping Centre (CEGEN, Barcelona). Genotype calling was done using the GeneTrain2.0 algorithm based on HapMap clusters implemented in the GenomeStudio Illumina software. The following quality control thresholds were applied: sample call rate>98% and/or logRRatio SD<0.3 (n=4 individuals were excluded in Valencia). Sex, relatedness (excluded: one duplicated sample and the younger brother of each of two brother-pairs detected in Sabadell), and heterozygosity were checked. Principal component analysis showed that there was no population stratification in the cohort. Genetic variants were filtered for a SNP call rate >95%, and minor allele frequency (MAF) >1%.

2.4 Neurodevelopment evaluation

In all birth cohorts, neurodevelopment of the children was assessed using Bayley Scales of Infant Development (BSID). The BSID assesses age-appropriate mental and psychomotor development expressed as Mental Development Index (MDI) and Psychomotor

Development Index (PDI), including performance abilities, memory, early language skills, psychomotor skills, and coordination.

In SCDS, BSID-II was used at 20 months of age (range: 15–25) for NC2 and 30 months of age (range: 27–35) for NC1. Testing was conducted at the Child Development Centre, Mahé by specially trained nurses. In INMA, the BSID-I was used around 14 months of age (range 11–20). All testing was done in the health care centre in the presence of the mother, by a total of 6 trained psychologists. In PHIME the BSID-III was used around the age of 18 months (range 16–20). Testing was conducted in the study hospitals in Italy and Greece by trained paediatricians or psychologists.

The different versions of the BSID utilize some different test items and are standardized on different normative populations. To homogenize the scales and allow us to directly compare associations, all raw scores were converted into standard deviation units (z-score equals raw score subtracted from mean and divided by the standard deviation) and then standardized to a mean of 100 and a standard deviation of 15 (new score = 100 + (15 × z)).

2.5 Other variables

Information about study population characteristics was obtained through questionnaires administered during pregnancy (19^{th}-36^{th} week) and at delivery in SCDS, during the first ($10–14^{th}$ week) and third ($28–32^{nd}$ week) trimester of pregnancy in INMA, during the third ($30–32^{nd}$ week) trimester of pregnancy in PHIME-Italy, and at delivery in PHIME-Greece. The variables used in this study were: maternal age at delivery (years), educational level (up to primary, secondary, university), fish intake during pregnancy (weekly servings), and tobacco consumption during pregnancy (no, yes). A socioeconomic status (SES) index was obtained in all cohorts. In SCDS, SES was measured as a continuous variable by the Hollingshead Four-Factor Socioeconomic Status scale modified for use in Seychelles (Davidson et al., 1998). In INMA, the parental social class was defined from the maternal or paternal occupation during pregnancy with the highest social class, according to a widely used Spanish adaptation of the International Standard Classification of Occupations approved in 1988 (ISCO88) coding system. In PHIME SES index was defined according to parental educational level, parental occupation (ISCO88), employment status during pregnancy (employed vs. not employed) and home ownership (Bennett et al., 2007). To homogenize this variable among the cohorts a three-category variable was calculated where the class I reflected the highest social class and the class III the lowest one.

2.6 Statistical analysis

Differences in THg concentrations (geometric means and 95% confidence intervals (CI)) in relation to genotype were analyzed by the Mann-Whitney test. When the frequency of variant homozygotes was low (<10%), variant homozygotes were pooled with heterozygotes. For further analysis the $\log_2$ of THg concentrations was calculated, due to its skewed distribution.

Linear regression models assessed the influence of CYP genotypes on the association between THg concentrations (in cord blood and maternal hair) and the BSID scores by including the interaction term $\log_2$-THg*genotype. The estimates for the association

between the scores and THg (both in cord blood and in maternal hair) within strata defined by genotypes were obtained for each country and were subsequently combined using a meta-analysis approach. In order to examine whether there was statistical heterogeneity, the I-squared measure was quantified (I^2) (Higgins et al., 2003). The fixed effects model was used and, if heterogeneity was detected (I^2>50%), we applied the random effects model. The interaction p-values were also provided. Firstly, models were adjusted for children's age at testing and child's sex (minimally adjusted models). Secondly, the models were adjusted by common variables among the cohorts (common variables adjusted models) that have been related to MDI and PDI in previous studies (Llop et al., 2012; Strain et al., 2015; Valent et al., 2013). Maternal fish consumption for the three cohorts was included in the minimally adjusted models to evaluate change in effect estimates. In all analyses the same reference genotype was used, i.e. genotypes with the higher activity allele.

Since the LD was low to moderate, the influence of the number of CYP3A variant alleles on the modifying effects on MDI and PDI was also evaluated. A four category genetic variable was built in order to evaluate any allele dosage effect (0: no variant alleles; 1: 1 variant allele; 2: 2 variant alleles; 3: 3 variant alleles). The analyses were carried out using the Stata, version 13, statistical package (StataCorp LP, College Station, Texas) and the R system version 3.0.

3. Results

Maternal and child characteristics are presented in Table 1. Mothers in NC1 and NC2 (Seychelles) were slightly younger than mothers in INMA-Spain and PHIME-Italy and Greece. The percentage of mothers with university degree was the lowest in PHIME-Greece, followed by INMA-Spain, PHIME-Italy, NC1 and NC2-Seychelles. Nearly half of the INMA-Spain mothers belonged to the lowest social class, followed by women in PHIME-Italy, PHIME-Greece, NC1 and NC2-Seychelles. The proportion of smokers was higher in the Mediterranean cohorts than in Seychelles. THg concentrations in maternal hair and cord blood were the highest in Seychelles (5.8 and 3.9 μg/g in NC1 & NC2 maternal hair respectively and 39.3 μg/L in NC1 cord blood). Among the Mediterranean cohorts, the highest cord blood THg concentrations were found in INMA-Spain (11.3 μg/L), followed by PHIME-Greece (7.5 μg/L) and finally PHIME-Italy (5.6 μg/L). The maternal fish consumption was higher in Seychelles (9.1 meals/week in NC1 and 8.5 meals/week in NC2), followed by INMA-Spain (6.2 meals/week), PHIME-Greece (3.2 meals/week) and Italy (2.5 meals/week). In all cohorts, the minor alleles of each CYP3A SNP correspond to the higher-activity allele with respect to predicted CYP enzyme activity (i.e., G in rs2257401 (*CYP3A7*), A in rs776746 (*CYP3A5*), and G in rs2740574 (*CYP3A4*)), with the exception of SCDS where higher-activity alleles A in rs776746 and G in rs2740574 were the major alleles (Table 2). Across all Mediterranean cohorts, the MAFs for all three CYP3A genes were relatively low (range from 1.4% to 12.4%), with minor cohort-differences in MAF for each SNP, and similar to reported MAFs for European populations (Supplementary Table S2). In contrast, in Seychelles (NC1 and NC2) the MAFs were much higher than the Mediterranean cohorts across all three CYP3A genes (range 44.8–55.1%, Table 2). These frequencies are similar to those previously reported for African populations and are somewhat higher than frequencies observed in Asian populations (Supplementary Table S2).

There was evidence of linkage disequilibrium (LD) between some of the SNPs: the LD (r^2) between rs776746 and rs2257401 were 0.39 in PHIME-Greece, 0.56 in PHIME-Italy, 0.60 in SCDS and 0.73 in INMA. The LD between all other SNP pairs was below 0.35. With respect to THg concentrations in cord blood, no significant differences were seen as a function of CYP3A genotype (Table 2).

Effect modification of CYP3A genotype on the association between prenatal exposure to MeHg and child neurodevelopment was evaluated. For *CYP3A7*, the overall coefficient of cord blood THg on MDI was positive (i.e. improving MDI score with increasing cord blood THg concentrations) and statistically significant (β= 2.90; 95%CI 1.53, 4.27) for children carrying GG or GC (Figure 1A). For children carrying CC, this coefficient became near null (β= 0.20; 95%CI −0.49, 0.89) (Figure 1B). The interaction between *CYP3A7* genotype and cord blood THg was statistically significant ($p<0.05$) for the Mediterranean cohorts. No effect modification of *CYP3A7* was evident for PDI (Figure 1C–D). For *CYP3A5*, the overall coefficient for the association between cord blood THg and MDI was positive for children carrying AA or AG (β= 2.51; 95%CI 1.04, 3.98) (Figure 2A) and near the null for children carrying GG (β= 0.40; 95%CI −2.27, 1.06) (Figure 2B). The interaction between cord blood THg and *CYP3A5* genotype was statistically significant ($p<0.05$) for INMA-Spain and PHIME-Greece. For PDI, the overall coefficient was positive but not statistically significant and the interaction was significant only for PHIME (Figure 2C–D). Results from the analysis of *CYP3A4* rs2740574 were similar to the *CYP3A7* and *CYP3A5* SNPs, but the interaction p-value in the MDI models was <0.05 for INMA only (Figure 3). Coefficients were virtually the same when we included maternal fish intake in the minimally adjusted models (Supplemental material Tables 3–5). Similar results were observed when we used the common variables adjusted models (Supplemental material Tables 6–8).

Effect modification of the three CYPA3 SNPs on the association between maternal hair mercury and child neuropsychological development was also evaluated. Results were more heterogeneous than for cord blood THg and we did not observe any evident genetic effect modification (Supplemental material Figures 1–3).

We also evaluated the influence of the number of CYP3A variant alleles (n=0 to 3) on the modifying effects on MDI and PDI. No clear allele dosage effect could be observed (data not shown).

4. Discussion

In this study of approximately 2,600 children born to mothers who consumed fish during pregnancy, we examined a potential role for genetic variation in CYP3A genes on the relationship between prenatal exposure to MeHg and child neurodevelopment. For all SNPs evaluated, there were no significant differences seen in THg levels in cord blood, indicating that CYP3A genotype, and presumably CYP activity, has little influence on toxicokinetics of MeHg in the fetus.

Prior reports for all four populations showed that an increase in prenatal Hg was not adversely associated with MDI (Davidson et al., 2008; Llop et al., 2012; Valent et al., 2013).

In this study, we found that for a doubling of cord blood THg concentrations, children carrying a high activity allele of CYP3A obtained higher MDI scores, and not children carrying low activity CYP3A alleles for whom coefficients obtained were near null. One explanation for improving scores with increasing THg might be that cord blood THg acts as a surrogate of fish nutrients that are beneficial for brain development, such as fatty acids, vitamins or selenium. Our findings suggest that the higher activity CYP3A alleles may modify the extent to which the benefits of such nutrients outweigh the adverse influences of mercury. Which nutrients would fall under a positive influence of CYP3A enzyme activity remains largely speculative; but a potential nutrient to be explored could be the vitamin D, which metabolism has been recently related with the human liver microsomal CYP3A4 (Cheng et al., 2016) and that has been associated with improved mental and psychomotor development in the Spanish cohort (Morales et al., 2012). Nevertheless, we observed similar results for the associations between CYP3A and neurodevelopmental scores in the sensitivity analysis by adjusting the models by fish consumption, which would indicate the existence of some residual confounding, and a more in-depth analysis evaluating specific fish-related biomarkers is needed to pinpoint which fish nutrients may influence the associations observed. Curiously, the strongest genetic effect modification was found in the Mediterranean cohorts where the CYP3A high-activity alleles occur with the lowest frequency, much lower than in the Seychelles cohorts where no significant effect modification was observed. This population level difference may suggest that there are population-specific genetic differences that influence the effect of CYP3A on mercury-related neurodevelopment. However, there could be other non-genetic factors that may explain these cohort-specific results. For example, the study population in Seychelles smoked less, included more high-level educated participants, and has a higher prenatal exposure to mercury. We tried to control for these factors in the multivariable analysis but we cannot discard the possibility of residual confounding.

We did not find any evidence of allele-dependent dosage effect for the CYP3A genes, implying that other polymorphisms in CYP3A or other genes explain the population differences. It is interesting to note that no effect of CYP3A genotype was found when maternal hair THg was used as marker of prenatal MeHg exposure. This could be due to differences in maternal hair sampling across the cohorts (hair length or pregnancy period). Hair THg levels have been shown to correlate highly with Hg levels in critical target tissues, such as the brain (Cernichiari et al., 1995) but we obtained strongest associations for cord blood THg which reflect more recent exposure in the third trimester of pregnancy. We speculate that, in this study, cord blood THg better reflect the fetal MeHg dose and thus is more relevant for studying interaction with the child's CYP3A genotype for neurodevelopmental outcomes.

In the populations we studied, children carrying high activity CYP3A alleles showed higher MDI scores as THg concentrations increased. This is consistent with an experimental study in fruit flies, where the transgenic expression of CYP6g1, or its human homolog *CYP3A4*, was seen to confer tolerance to MeHg toxicity during development (Rand et al., 2012). However, the mechanism for this tolerance to toxicity remains uncertain. In humans, the CYP3A enzymes are known to be involved in the metabolism of xenobiotics primarily in the liver but also in extrahepatic tissues including placenta, kidney, intestines, and lung (Pavek

and Dvorak, 2008). Consistent with the notion that CYP3A genes afford MeHg protection during development, we observed that the strongest association occurred between MeHg and *CYP3A7*, which is the CYP3A isoform that is expressed exclusively in the fetal liver (Stevens et al., 2003). Polymorphisms in the CYP3A genes are known to alter the metabolism of some xenobiotics (Pavek and Dvorak, 2008). Since cord blood THg concentrations were not seen to vary as a function of CYP3A genotype, it appears that CYP3A enzymes have little influence on MeHg toxicokinetics. An alternative explanation for the beneficial effects of more active CYP3A genotypes may relate to metabolism and clearance of toxic by-products of a MeHg insult. In this regard, CYP3A4 catalyzes the reduction of α- and β-unsaturated aldehydes, notably, 4-hydroxynonenal (4-HNE) (Amunom et al., 2011), a common endogenous product of lipid peroxidation resulting from metal toxicity (Valko et al., 2005). Localized expression of CYP3A activity, e.g. in the brain, may therefore have a neuroprotective function, and accordingly, reduced CYP activity, via MeHg inhibition, could leave the brain vulnerable. In support of this notion, inhibition of CYP3A4 or CYP2D6 activity in SH-SY5Y human neuroblastoma cells has been shown to enhance the neurotoxicity of MPP+ (1-methyl-4-phenylpyridinium), a neurotoxic derivative of MPTP ((1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and an agent used to induce dopaminergic neuron death in models of Parkinson's disease (Mann and Tyndale, 2010). Resolving mechanisms by which CYP enzymes alleviate toxic effects specific to MeHg will require further studies.

Polymorphisms in the CYP3A genes are known to alter the metabolism of some xenobiotics (Pavek and Dvorak, 2008). Thus, the notion that CYPs could mediate MeHg biotransformation (de-methylation) in the liver presents an attractive hypothesis. However, cord blood THg concentrations were not seen to vary as a function of CYP3A genotype, indicating CYP3A enzymes have little influence on MeHg toxicokinetics. An alternative explanation for the beneficial effects of more active CYP3A genotypes may relate to metabolism of by products of the MeHg insult. In this regard, CYP3A4 catalyzes the reduction of α- and β-unsaturated aldehydes, notably, 4-hydroxynonenal (4-HNE) (Amunom et al., 2011), a common endogenous product of lipid peroxidation resulting from metal toxicity (Valko et al., 2005). Localized expression of CYP3A activity, for example in the brain, may therefore elicit a neuroprotective function. Resolving mechanisms by which CYP enzymes might participate in MeHg metabolism and elimination, or alternatively alleviate MeHg toxic effects, will require further studies.

The main strengths of our study are the multi-cohort design and the large study population. Furthermore, we compared different populations that were all exposed to MeHg through fish consumption. Additionally, children's neurodevelopment was assessed prospectively using standardized and validated neuropsychological tests and questionnaires. The main limitation of our study was the heterogeneity in the measure of neurodevelopment since different editions of the Bayley test were used. Also slightly different child age ranges were considered. We addressed these limitations by homogenizing the scales and using a meta-analysis approach.

In conclusion, the magnitude of the association between prenatal exposure to MeHg and child neuropsychological development appears to be modified by polymorphisms in

CYP3A7 and *CYP3A5* genes in some of the populations studied. These results provide some support for the hypothesis that CYP3A genes may modify the response to MeHg-containing fish exposure during early life development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Amunom I, Dieter LJ, Tamasi V, Cai J, Conklin DJ, Srivastava S, Martin MV, Guengerich FP, Prough RA. Cytochromes P450 catalyze the reduction of α,β-unsaturated aldehydes. Chem Res Toxicol. 2011; 24:1223–1230. [PubMed: 21766881]

Anzenbacher P, Anzenbacherová E. Cytochromes P450 and metabolism of xenobiotics. Cell Mol Life Sci. 2001; 58:737–747. [PubMed: 11437235]

Ballatori N, Clarkson TW. Biliary transport of glutathione and methylmercury. Am J Physiol. 1983; 244:G435–441. [PubMed: 6837749]

Barcelos GR, Grotto D, de Marco KC, Valentini J, Lengert A, de Oliveira AA, Garcia SC, Braga GU, Schlawicke EK, Colus IM, Broberg K, Barbosa F Jr. Polymorphisms in glutathione-related genes modify mercury concentrations and antioxidant status in subjects environmentally exposed to methylmercury. Sci Total Environ. 2013; 463–464:319–325.

Bennett GG, Wolin KY, James SA. Lifecourse socioeconomic position and weight change among blacks: The Pitt County study. Obesity (Silver Spring). 2007; 15:172–181. [PubMed: 17228045]

Cernichiari E, Myers GJ, Ballatori N, Zareba G, Vyas J, Clarkson T. The biological monitoring of prenatal exposure to methylmercury. Neurotoxicology. 2007; 28:1015–1022. [PubMed: 17382399]

Cernichiari E, Toribara TY, Liang L, Marsh DO, Berlin MW, Myers GJ, Cox C, Shamlaye CF, Choisy O, Davidson P. The biological monitoring of mercury in the Seychelles study. Neurotoxicology. 1995; 16:613–628. [PubMed: 8714867]

Cheng CYS, Slominski AT, Tuckey RC. Hydroxylation of 20-hydroxyvitamin D3 by human CYP3A4. J Steroid Biochem Mol Biol. 2016; 159:131–141. [PubMed: 26970587]

Custodio HM, Broberg K, Wennberg M, Jansson JH, Vessby B, Hallmans G, Stegmayr B, Skerfving S. Polymorphisms in glutathione-related genes affect methylmercury retention. Arch Environ Health. 2004; 59:588–595. [PubMed: 16599007]

Davidson PW, Strain JJ, Myers GJ, Thurston SW, Bonham MP, Shamlaye CF, Stokes-Riner A, Wallace JM, Robson PJ, Duffy EM, Georger LA, Sloane-Reeves J, Cernichiari E, Canfield RL, Cox C, Huang LS, Janciuras J, Clarkson TW. Neurodevelopmental effects of maternal nutritional status and exposure to methylmercury from eating fish during pregnancy. Neurotoxicology. 2008; 29:767–775. [PubMed: 18590763]

Engström K, Love TM, Watson GE, Zareba G, Yeates A, Wahlberg K, Alhamdow A, Thurston SW, Mulhern M, McSorley EM, Strain JJ, Davidson PW, Shamlaye CF, Myers GJ, Rand MD, van

Wijngaarden E, Broberg K. Polymorphisms in ATP-binding cassette transporters associated with maternal methylmercury disposition and infant neurodevelopment in mother-infant pairs in the Seychelles Child Development Study. Environ Int. 2016; 94:224–229. [PubMed: 27262785]

Engstrom K, Stromberg U, Lundh T, Johansson I, Vessby B, Hallmans G, Skerfving S, Broberg K. Genetic variation in glutathione-related genes and body burden of methylmercury. Environ Health Perspect. 2008; 116:734–739. [PubMed: 18560528]

Farris FF, Dedrick RL, Allen PV, Smith JC. Physiological model for the pharmacokinetics of methyl mercury in the growing rat. Toxicol Appl Pharmacol. 1993; 119:74–90. [PubMed: 8470126]

Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sorensen N, Dahl R, Jorgensen PJ. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol. 1997; 19:417–428. [PubMed: 9392777]

Gundacker C, Komarnicki G, Jagiello P, Gencikova A, Dahmen N, Wittmann KJ, Gencik M. Glutathione-S-transferase polymorphism, metallothionein expression, and mercury levels among students in Austria. Sci Total Environ. 2007; 385:37–47. [PubMed: 17716707]

Guxens M, Ballester F, Espada M, Fernandez MF, Grimalt JO, Ibarluzea J, Olea N, Rebagliato M, Tardon A, Torrent M, Vioque J, Vrijheid M, Sunyer J. Cohort Profile: the INMA--INfancia y Medio Ambiente--(Environment and Childhood) Project. Int J Epidemiol. 2012; 41:930–940. [PubMed: 21471022]

Hakkola J, Pelkonen O, Pasanen M, Raunio H. Xenobiotic-metabolizing cytochrome P450 enzymes in the human feto-placental unit: role in intrauterine toxicity. Crit Rev Toxicol. 1998; 28:35–72. [PubMed: 9493761]

Hasler JA. Pharmacogenetics of cytochromes P450. Mol Aspects Med. 1999; 20:12–137. [PubMed: 10575648]

Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003; 327:557–560. [PubMed: 12958120]

Johansson I, Ingelman-Sundberg M. Genetic polymorphism and toxicology--with emphasis on cytochrome p450. Toxicol Sci. 2011; 120:1–13. [PubMed: 21149643]

Knops N, van den Heuvel LP, Masereeuw R, Bongaers I, de LH, Levtchenko E, Kuypers D. The functional implications of common genetic variation in CYP3A5 and ABCB1 in human proximal tubule cells. Mol Pharm. 2015; 12:758–768. [PubMed: 25590378]

Lamba JK, Lin YS, Schuetz EG, Thummel KE. Genetic contribution to variable human CYP3A-mediated metabolism. Adv Drug Deliv Rev. 2002; 54:1271–1294. [PubMed: 12406645]

Llop S, Ballester F, Broberg K. Effect of Gene-Mercury Interactions on Mercury Toxicokinetics and Neurotoxicity. Curr Envir Health Rpt. 2015; 2:179–9.

Llop S, Guxens M, Murcia M, Lertxundi A, Ramon R, Riano I, Rebagliato M, Ibarluzea J, Tardon A, Sunyer J, Ballester F. Prenatal Exposure to Mercury and Infant Neurodevelopment in a Multicenter Cohort in Spain: Study of Potential Modifiers. Am J Epidemiol. 2012; 175:451–465. [PubMed: 22287639]

Llop S, Guxens M, Murcia M, Lertxundi A, Ramon R, Riaño I, Rebagliato M, Ibarluzea J, Tardon A, Sunyer J, Ballester F. INMA Project. Prenatal exposure to mercury and infant neurodevelopment in a multicenter cohort in Spain: study of potential modifiers. Am J Epidemiol. 2012; 175:451–465. [PubMed: 22287639]

Mahapatra CT, Bond J, Rand DM, Rand MD. Identification of methylmercury tolerance gene candidates in Drosophila. Toxicol Sci. 2010; 116:225–238. [PubMed: 20375079]

Mann A, Tyndale RF. Cytochrome P450–2D6 enzyme neuroprotects against 1-methyl-4-phenylpyridinium toxicity in SH-SY5Y neuronal cells. Eur J Neurosci. 2010; 31(7):1185–93. [PubMed: 20345925]

Miklavcic A, Casetta A, Snoj TJ, Mazej D, Krsnik M, Mariuz M, Sofianou K, Spiric Z, Barbone F, Horvat M. Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranean area. Environ Res. 2013; 120:7–17. [PubMed: 22999706]

Morales E, Guxens M, Llop S, Rodríguez-Bernal CL, Tardón A, Riaño I, Ibarluzea J, Lertxundi N, Espada M, Rodriguez A, Sunyer J. INMA Project. Circulating 25-hydroxyvitamin D3 in pregnancy and infant neuropsychological development. Pediatrics. 2012; 130:e913–920. [PubMed: 22987876]

Nakayama M. Biotransformation of methylmercury with special reference to hepatic microsomal cytochrome P-450 linked monooxygenase system. Kumamoto Med J. 1976; 29:95–109. [PubMed: 1011794]

National Research Council. Scientific frontiers in developmental toxicology and risk assessment. Washington, DC: 2000.

Parks JM, Johs A, Podar M, Bridou R, Hurt RA Jr, Smith SD, Tomanicek SJ, Qian Y, Brown SD, Brandt CC, Palumbo AV, Smith JC, Wall JD, Elias DA, Liang L. The genetic basis for bacterial mercury methylation. Science. 2013; 339:1332–1335. [PubMed: 23393089]

Pavek P, Dvorak Z. Xenobiotic-induced transcriptional regulation of xenobiotic metabolizing enzymes of the cytochrome P450 superfamily in human extrahepatic tissues. Curr Drug Metab. 2008; 9:129–143. [PubMed: 18288955]

Ramon R, Murcia M, Aguinagalde X, Amurrio A, Llop S, Ibarluzea J, Lertxundi A, varez-Pedrerol M, Casas M, Vioque J, Sunyer J, Tardon A, Martinez-Arguelles B, Ballester F. Prenatal mercury exposure in a multicenter cohort study in Spain. Environ Int. 2011; 37:597–604. [PubMed: 21239061]

Rand MD, Lowe JA, Mahapatra CT. Drosophila CYP6g1 and its human homolog CYP3A4 confer tolerance to methylmercury during development. Toxicology. 2012; 300:75–82. [PubMed: 22699155]

Rodriguez-Antona C, Jande M, Rane A, Ingelman-Sundberg M. Identification and phenotype characterization of two CYP3A haplotypes causing different enzymatic capacity in fetal livers. Clin Pharmacol Ther. 2005; 77:259–270. [PubMed: 15903124]

Smith JC, Allen PV, Turner MD, Most B, Fisher HL, Hall LL. The kinetics of intravenously administered methyl mercury in man. Toxicol Appl Pharmacol. 1994; 128:251–256. [PubMed: 7940540]

Stevens JC, Hines RN, Gu C, Koukouritaki SB, Manro JR, Tandler PJ, Zaya MJ. Developmental expression of the major human hepatic CYP3A enzymes. J Pharmacol ExpTher. 2003; 307:573–582.

Strain JJ, Yeates AJ, van WE, Thurston SW, Mulhern MS, McSorley EM, Watson GE, Love TM, Smith TH, Yost K, Harrington D, Shamlaye CF, Henderson J, Myers GJ, Davidson PW. Prenatal exposure to methyl mercury from fish consumption and polyunsaturated fatty acids: associations with child development at 20 mo of age in an observational study in the Republic of Seychelles. Am J Clin Nutr. 2015; 101:530–537. [PubMed: 25733638]

Valent F, Mariuz M, Bin M, Little D, Mazej D, Tognin V, Tratnik J, McAfee AJ, Mulhern MS, Parpinel M, Carrozzi M, Horvat M, Tamburlini G, Barbone F. Associations of prenatal mercury exposure from maternal fish consumption and polyunsaturated fatty acids with child neurodevelopment: a prospective cohort study in Italy. J Epidemiol. 2013; 23:360–370. [PubMed: 23933621]

Valko M, Morris H, Cronin MTD. Metals, toxicity and oxidative stress. Curr Med Chem. 2005; 12:1161–1208. [PubMed: 15892631]

World Health Organization (WHO). Health risks of heavy metals from long-range transboundary air pollution. 2007a.

World Health Organization (WHO). Exposure to mercury: a major public health concern. 2007b.

Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther. 2013; 138:103–141. [PubMed: 23333322]

Highlights

- Genetics may influence the neurotoxicity of MeHg
- The association between cord blood Hg and children's neurodevelopment was modified by variations in the *CYP3A5* and *CYP3A7* genes in three birth cohorts
- *CYP3A* genes may influence the response to MeHg exposure during early life development

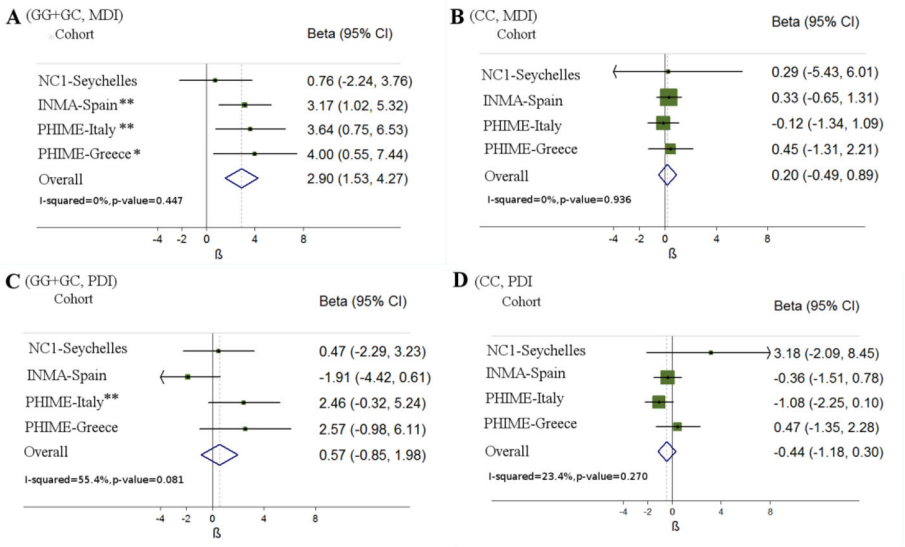

Figure 1.

Meta-analysis of the association between cord blood mercury concentrations and MDI and PDI scores according to the child genotype for *CYP3A7* rs2257401. A) Association between cord blood mercury and MDI scores for children with the GG+GC genotype. B) Association between cord blood mercury and MDI scores for children with the CC genotype. C) Association between cord blood mercury and PDI scores for children with the GG+GC genotype. D) Association between cord blood mercury and PDI scores for children with the CC genotype

G is the allele with the highest activity

Models adjusted by sex and age at testing (also by subcohort for INMA)

Random effects were applied if heterogeneity (I-squared)>50%

CI: Confidence intervals

*Interaction p-value<0.1 **Interaction p-value<0.05

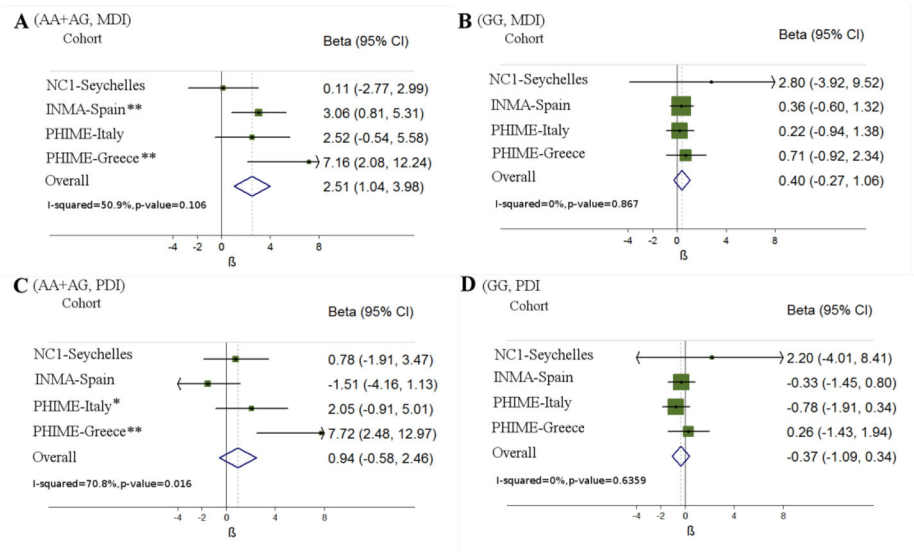

Figure 2.

Meta-analysis of the association between cord blood mercury concentrations and MDI and PDI scores according to the child genotype for *CYP3A5* rs776746. A) Association between cord blood mercury and MDI scores for children with the AA+AG genotype. B) Association between cord blood mercury and MDI scores for children with the GG genotype. C) Association between cord blood mercury and PDI scores for children with the AA+AG genotype. D) Association between cord blood mercury and PDI scores for children with the GG genotype

A is the allele with the highest activity

Models adjusted by sex and age at testing (also by subcohort for INMA)

Random effects were applied if heterogeneity (I-squared)>50%

CI: Confidence intervals

*Interaction p-value<0.1 **Interaction p-value<0.05

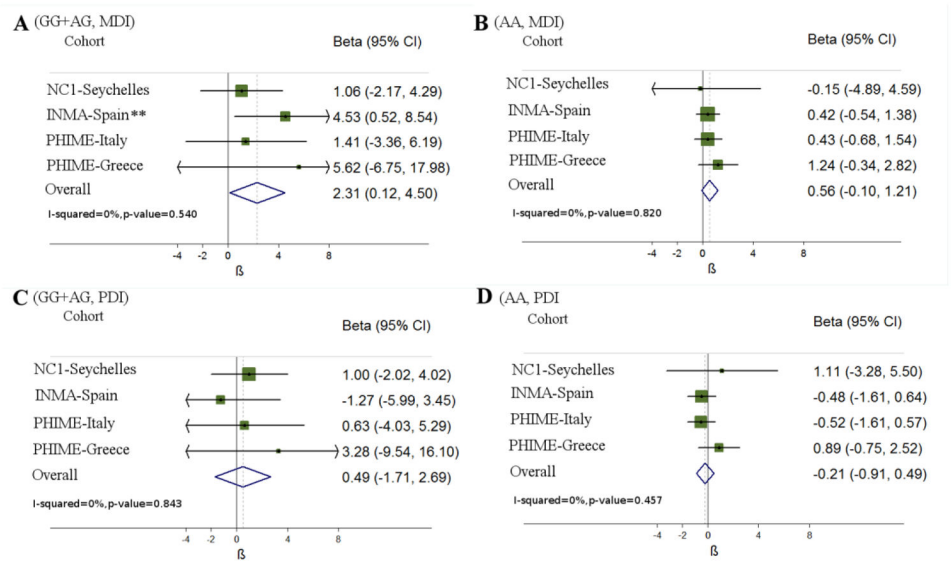

Figure 3.

Meta-analysis of the association between cord blood mercury concentrations and MDI and PDI scores according to the child genotype for *CYP3A4* rs2740574. A) Association between cord blood mercury and MDI scores for children with the GG+AG genotype. B) Association between cord blood mercury and MDI scores for children with the AA genotype. C) Association between cord blood mercury and PDI scores for children with the GG+AG genotype. D) Association between cord blood mercury and PDI scores for children with the AA genotype

G is the allele with the highest activity

Models adjusted by sex and age at testing (also by subcohort for INMA)

Random effects were applied if heterogeneity (I-squared)>50% CI: Confidence intervals

*Interaction p-value<0.1 **Interaction p-value<0.05

Table 1

Distribution of maternal and child characteristics.

	NC1-Seychelles (%)	NC2-Seychelles (%)	INMA-Spain (%)	PHIME-Italy (%)	PHIME-Greece (%)	p-value[3]
Total	211	949	625	573	281	
Maternal age at delivery (years) [1]	27.8 (6.0)	27.0 (6.3)	31.2 (4.3)	33.3 (4.4)	28.7 (6.4)	<0.001
Maternal education						
Up to primary	0.5	0.4	30.2	17.3	22.8	<0.001
Secondary	51.2	41.1	42.2	48.3	56.3	
University	48.3	58.1	27.5	34.4	20.9	
Social class						
High	37.9	27.6	28.5	27.4	39.0	<0.001
Medium	49.8	60.8	31.0	28.9	18.9	
Low	12.3	11.6	40.5	43.7	42.0	
Smoking habit during prenancy						<0.001
Smokers	2.8	0.8	18.5	11.6	16.6	
Children's sex						0.278
Female	51.2	49.2	47.4	48.4	55.3	
Children age at testing (months) [1]	27.9 (1.2)	20.3 (1.3)	13.4 (1.3)	18.7 (0.9)	18.5 (1.1)	<0.001
Maternal fish consumption (meals/week) [1]	9.1 (4.1)	8.5 (4.7)	6.2 (2.6)	2.5 (1.6)	3.2 (2.3)	
THg concentrations in maternal hair (μg/g) [1,2]	5.8 (3.7)	3.9 (3.5)	na	1.0 (1.0)	1.4 (1.1)	<0.001
THg concentrations in cord blood (μg/L) [1,2]	39.3 (25.5)	na	11.3 (9.8)	5.6 (4.8)	7.5 (5.8)	<0.001

[1] mean (standard deviation)

[2] Italy: n=453 for THg in cord blood and n=571 for THg in maternal hair

[3] Kruskal Wallis test for continuous variables and Chi^2 for categorical variables

Na: not available

THg: total mercury

SCDS-NC: The Seychelles Child Development Study Nutrition Cohort

INMA: Infancia y medio ambiente (i.e. Environment and Childhood)

PHIME: Public Health Impact of long-term, low-level Mixed Element Exposure in susceptible population strata

Table 2

Genetic characteristics of the CYP3A single nucleotide polymorphisms and cord blood mercury levels (geometric means and 95% confidence intervals) according to genotype[1].

Cohort	SNPs	Gene[2]	Genotype	N	Genotype (%)	MAF (%)	p-value HW	Hg GM (μg/L)	CI 95%	P-value[4]
NC1- Seychelles	rs2257401	*CYP3A7*	GG+GC	145	68.7	44.8	0.906	36.19	32.97–39.72	0.526
			CC[3]	66	31.3			38.15	34.18–42.59	
	rs776746	*CYP3A5*	AA+AG	167	79.1	54.5	0.813	36.27	33.32–39.47	0.568
			GG[3]	44	20.9			38.75	33.78–44.46	
	rs2740574	*CYP3A4*	GG+AG	141	68.1	54.1	0.477	37.34	34.28–40.68	0.551
			AA[3]	66	31.9			36.26	31.40–41.86	
NC2- Seychelles	rs2257401	*CYP3A7*	GG+GC	678	71.7	46.5	0.906			
			CC	268	28.3					
	rs776746	*CYP3A5*	AA+AG	756	79.7	55.1	0.813		Na	
			GG	193	20.3					
	rs2740574	*CYP3A4*	GG+AG	667	70.8	54.0	0.477			
			AA	275	29.2					
INMA-Spain	rs2257401	*CYP3A7*	GG+GC	123	18.2	9.5	0.948	8.48	7.40–9.73	0.711
			CC	554	81.8			8.21	7.67–8.78	
	rs776746	*CYP3A5*	AA+AG	104	15.1	7.8	0.535	8.07	6.93–9.41	0.664
			GG	583	84.9			8.30	7.77–8.87	
	rs2740574	*CYP3A4*	GG+AG	43	6.8	3.5	0.776	9.86	7.94–12.23	0.201
			AA	593	93.2			8.22	7.70–8.78	
PHIME-Italy	rs2257401	*CYP3A7*	GG+GC	66	15.1	7.8	0.664	3.84	3.09–4.79	0.701
			CC	370	84.9			3.91	3.57–4.29	
	rs776746	*CYP3A5*	AA+AG	51	11.2	5.7	0.672	3.82	2.92–5.00	0.675
			GG	404	88.8			3.95	3.62–4.31	
	rs2740574	*CYP3A4*	GG+AG	29	6.3	3.2	0.484	4.50	3.30–6.13	0.711
			AA	429	93.7			3.90	3.58–4.25	
PHIME- Greece	rs2257401	*CYP3A7*	GG+GC	63	23.2	12.4	0.937	5.71	4.70–6.94	0.679
			CC	208	76.8			5.43	4.84–6.09	
	rs776746	*CYP3A5*	AA+AG	33	11.7	5.9	0.295	5.99	4.62–7.77	0.556

Cohort	SNPs	Gene[2]	Genotype	N	Genotype (%)	MAF (%)	p-value HW	Hg GM (μg/L)	CI 95%	P-value[4]
			GG	248	88.3			5.54	5.00–6.14	
	rs2740574	*CYP3A4*	GG+AG	8	2.8	1.4	0.808	5.08	2.98–8.67	0.556
			AA	273	97.2			5.61	5.09–6.18	

[1] Alleles with higher activity: G in rs2257401, A in rs776746, and G in rs2740574.

[2] All three CYP3A genes are located on chromosome 7. Chromosome bands: 7q21.1 for *CYP3A5,* 7q22.1 for *CYP3A7,* 7q21.1 for *CYP3A4*

[3] Homozygote for the minor allele

[4] Refers to group differences in mercury levels (Mann-Whitney test)

CI: Confidence intervals

GM: geometric mean

HW: Hardy Weinberg equilibrium

MAF: minor allele frequency (for the allele that is minor allele in the majority of populations under study).