Environment International 115 (2018) 142–149

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Maternal polymorphisms in glutathione-related genes are associated with maternal mercury concentrations and early child neurodevelopment in a population with a fish-rich diet

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ARTICLE INFO

Handling Editor: Olga-Ioanna Kalantzi

Keywords:
Methylmercury
GCLC
GCLM
GSTP1
Neurodevelopment

ABSTRACT

Introduction: Glutathione (GSH) pathways play a key role the metabolism and elimination of the neurotoxicant methylmercury (MeHg). We hypothesized that maternal genetic variation linked to GSH pathways could influence MeHg concentrations in pregnant mothers and children and thereby also affect early life development.
Methods: The *GCLM* (rs41303970, C/T), *GCLC* (rs761142, T/G) and *GSTP1* (rs1695, A/G) polymorphisms were genotyped in 1449 mothers in a prospective study of the Seychellois population with a diet rich in fish. Genotypes were analyzed in association with maternal hair and blood Hg, fetal blood Hg (cord blood Hg), as well as children's mental (MDI) and motor development (PDI; MDI and PDI assessed by Bayley Scales of Infant Development at 20 months). We also examined whether genotypes modified the association between Hg exposure and developmental outcomes.
Results: *GCLC* rs761142 TT homozygotes showed statistically higher mean maternal hair Hg (4.12 ppm) than G carriers (AG 3.73 and GG 3.52 ppm) ($p = 0.037$). For the combination of *GCLC* rs761142 and *GCLM* rs41303970, double homozygotes TT + CC showed higher hair Hg (4.40 ppm) than G + T carriers (3.44 ppm; $p = 0.018$). No associations were observed between *GSTP1* rs1695 and maternal hair Hg or between any genotypes and maternal blood Hg or cord blood Hg. The maternal *GSTP1* rs1695 rare allele (G) was associated with a lower MDI among children ($\beta = -1.48$, $p = 0.048$). We also observed some interactions: increasing Hg in maternal and cord blood was associated with lower PDI among *GCLC* rs761142 TT carriers; and increasing Hg in hair was associated with lower MDI among *GSTP1* rs1695 GG carriers.
Conclusions: Maternal genetic variation in genes involved in GSH synthesis is statistically associated with Hg concentrations in maternal hair, but not in maternal or fetal blood. We observed interactions that suggest maternal GSH genetics may modify associations between MeHg exposure and neurodevelopmental outcomes.

1. Introduction

Fish is the main source of human low-level methylmercury (MeHg) exposure. At high levels, MeHg has clear detrimental effects on the nervous system (Clarkson et al., 2003), but the neurotoxic effects of low-level exposure are not established. The developing brain is particularly sensitive to neurotoxicants including MeHg (Costa et al., 2004; Johansson et al., 2007), but it is unclear at what MeHg level the fetal brain is affected. Consequently, it is unclear if fish ingestion poses a risk for fetal toxicity. Research results of MeHg exposure from fish consumption in relation to neurodevelopmental outcomes in children have been contradictory between studies of different populations, with adverse associations observed in some studies (Grandjean et al., 1997; Vejrup et al., 2016), but not in others (Daniels et al., 2004; Davidson

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https://doi.org/10.1016/j.envint.2018.03.015
Received 28 December 2017; Received in revised form 11 March 2018; Accepted 11 March 2018

et al., 1998; Llop et al., 2012; Strain et al., 2015). Several studies have suggested that genetics may contribute to MeHg body burden as well as to defense mechanisms against MeHg toxicity (Andreoli and Sprovieri, 2017; Llop et al., 2015).

An important mechanism in MeHg metabolism involves the conjugation of MeHg to the small tripeptide glutathione (GSH), which facilitates elimination of the conjugate in the bile via the ABC-transporter system (Ballatori and Clarkson, 1985). The rate-limiting enzyme for GSH synthesis is γ-glutamyl-cysteine ligase (GCL), which is composed of a catalytic subunit (GCLC) and a modifier subunit (GCLM) (Lu, 2013). Further, the conjugation of GSH to MeHg has been suggested to be catalyzed by glutathione S-transferases, particularly the pi 1 isoform (GSTP1) (Custodio et al., 2004). Genetic polymorphisms in *GCLC*, *GCLM*, and *GSTP1* have been linked to MeHg retention and body burden in adults (Barcelos et al., 2013; Custodio et al., 2004; Goodrich et al., 2011; Parajuli et al., 2016; Schlawicke Engstrom et al., 2008). In addition, our group has recently shown that GSTP1 polymorphisms, expressed in *Drosophila*, may influence MeHg toxicity during development through both toxicokinetic and toxicodynamic mechanisms (Vorojeikina et al., 2017).

Accordingly, we hypothesized that maternal polymorphisms in the GSH pathway could modify maternal MeHg body burden, and thereby influence MeHg exposure in the fetus and, as a consequence influence early child neurodevelopment. We have genotyped maternal SNPs in *GCLM*, *GCLC* and *GSTP1* in 1449 pregnant women from a population in the Seychelles with a diet rich in fish and in whom no consistent adverse associations between maternal MeHg exposure and neurodevelopment were observed in their children (Strain et al., 2015; Strain et al., 2012; van Wijngaarden et al., 2017). SNPs were analyzed in association with MeHg biomarker concentrations in mothers (hair and blood) and children (cord blood), as well as early neurodevelopmental outcomes in children (Bayley scales of infant development; BSID). The influence of an interaction between SNPs and biomarkers of MeHg exposure upon neurodevelopment endpoints was also studied, since antioxidative effects of glutathione may be protective against oxidative stress generated by MeHg (Kaur et al., 2006).

2. Materials and methods

2.1. Study population

This prospective cohort consists of mother-child pairs from the Republic of Seychelles in the Indian Ocean and is of mixed African, European and East Asian origin. Participants were recruited for the Seychelles Child Development Study (SCDS) Nutrition Cohort 2 (NC2), a longitudinal observational study with the overall aim to investigate the effects of MeHg exposure from maternal fish consumption during pregnancy, nutritional status, and genetic predisposition on child developmental outcomes. NC2 consists of 1535 apparently healthy mothers recruited between the years 2008 to 2011 during their first antenatal visit (from 14 weeks of gestation) at eight health centers across the main Island Mahé. Inclusion criteria for NC2 included being native Seychellois, being ≥16 y of age, having a singleton pregnancy, and having no obvious health concerns. Further information on recruitment criteria and power calculations for NC2 has previously been described (Strain et al., 2015). Mothers completed a retrospective fish use questionnaire at 28 weeks gestation, to estimate their weekly consumption of fish during pregnancy. Non-fasting blood samples were collected at 28 weeks gestation, and cord blood and maternal hair were collected at delivery. Whole blood samples were processed at the Public Health Laboratory at the Ministry of Health. One aliquot was shipped to the University of Rochester for Hg analysis and a second aliquot was shipped to Lund University for genotyping.

For prenatal biomarker analyses, participants without genetic data and one each of thirty sibling pairs were excluded; also, missing data varied for the three biomarkers. (A flow chart of the participants included in this study is presented in Supplemental Fig. S1). DNA from blood for genotyping was available for 1449 mothers. DNA and biomarker values were available for 1311, 1379, and 1004 mother child pairs for hair, maternal blood, and cord blood, respectively. For the BSID endpoints, exclusions were determined as described in Strain et al. (2015) and included pre- or perinatal deaths, maternal pre- or perinatal complications, birthweight < 1500 g, head trauma, twin births and seizures or disability. Additionally, participants without genetic data and one each of thirteen sibling pairs were excluded. There were 1330 pairs eligible for models for the BSID endpoints, 1230, 1266, and 935 of whom had samples of hair, maternal and cord blood respectively. The study was conducted according to guidelines laid down in the Declaration of Helsinki and all study procedures involving participants were reviewed and approved by the Seychelles Ethics Board, the Research Subjects Review Board at the University of Rochester, and the Regional Ethics Committee at Lund University, Sweden.

2.2. Hg measurements

Hair samples were cut at delivery and the longest available segment of maternal hair growing during gestation was analyzed assuming a hair growth rate of 1.1 cm/month. Total mercury in maternal hair during gestation is an established biomarker for prenatal MeHg exposure and has been used to monitor neurotoxicity of methylmercury; maternal hair Hg is known to correlate with infant brain Hg levels (Cernichiari et al., 1995) and is believed to reflect the species of Hg that is transported across the blood-brain barrier (Clarkson and Magos, 2006). Total Hg in hair was measured by cold-vapor atomic-absorption-spectrometry (CVAAS) as previously described (Cernichiari et al., 1995) and reported in parts per million (ppm). Total Hg was measured on stored maternal and cord whole blood samples with atomic fluorescence spectrometry using a PSA Millennium Merlin System (PS Analytical, Kent, UK). The limit of detection for THg in maternal hair was 0.14 ppm and our limit of detection for Hg in blood was approximately 0.01 ng/mL, depending on sample volume (Pichichero et al., 2008).

2.3. Neurodevelopmental assessment

Toddlers completed developmental testing with the Bayley Scales of Infant Development (BSID-II) at 20 months (range: 15–32 months). The BSID-II yields two scores, the Mental Developmental Index (MDI) and the Psychomotor Developmental Index (PDI). Both scores are standardized with a Mean = 100 and an SD = 15. Testing was conducted by specially trained nurses at the Child Development Centre, Mahé. All study forms were shipped to the University of Rochester, where data were double-entered. Test reliabilities for the BSID-II were determined as previously described (Strain et al., 2008).

2.4. Genetic analyses

In this study, we selected genes encoding proteins with an important role in the GSH pathway for metabolising toxicants, including MeHg: two genes (*GCLC* and *GCLM*) encoding the rate-limiting enzyme for the synthesis of GSH (Lu, 2013) as well as glutathione S-transferase (*GSTP1*) respectively. The latter enzyme has been suggested to conjugate MeHg to GSH (Custodio et al., 2004). The selected SNPs included rs761142 (*GCLC*), rs41303970 (*GCLM*) and rs1695 (*GSTP1*) and are presented in Table 1. SNPs were selected based on a careful review of published literature (Llop et al., 2015) and we included only SNPs that had been shown to influence expression/regulation of the corresponding gene (i.e. rs761142 in *GCLC* and rs41303970 in *GCLM*) and/or main effect associations with Hg biomarker concentrations (i.e. rs41303970 in *GCLM* and rs1695 in *GSTP1*). In addition SNPs were selected with consideration to previously reported minor allele frequencies (MAFs) of relevant populations (i.e. African, Asian and European populations) and only SNPs with MAFs > 5% were included in