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Contribution of child ABC-transporter genetics to prenatal MeHg exposure and neurodevelopment

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Abstract

Background: There is emerging evidence that exposure to prenatal methylmercury (MeHg) from maternal fish consumption during pregnancy can differ between individuals due to genetic variation. In previous studies, we have reported that maternal polymorphisms in ABC-transporter genes were associated with maternal hair MeHg concentrations, and with children's early neurodevelopmental tests. In this study, we add to these findings by evaluating the contribution of genetic variation in children's ABC-transporter genes to prenatal MeHg exposure and early child neurodevelopmental tests.

Methods—We genotyped six polymorphisms (rs2032582, rs10276499 and rs1202169 in *ABCB1*; rs11075290 and rs215088 in *ABCC1*; rs717620 in *ABCC2*) in DNA from cord blood and maternal blood of the Seychelles Child Development Study Nutrition Cohort 2. We determined prenatal MeHg exposure by measuring total mercury (Hg) in cord blood by atomic fluorescence spectrometry. We assessed neurodevelopment in children at approximately 20 months using the Bayley Scales of Infant Development (BSID-II). We used linear regression models to analyze covariate-adjusted associations of child genotype with cord MeHg and BSID-II outcomes (Mental Developmental and Psychomotor Developmental Indexes). We also evaluated interactions between genotypes, cord MeHg, and neurodevelopmental outcomes. All models were run with and without adjustment for maternal genotype.

Results: Of the six evaluated polymorphisms, only *ABCC1* rs11075290 was associated with cord blood MeHg; children homozygous for the T-allele had on average 29.99 μg/L MeHg in cord blood while those homozygous for the C-allele had on average 38.06 μg/L MeHg in cord blood ($p<0.001$). No polymorphisms in the children were associated with either subscale of the BSID. However, the association between cord MeHg and the Mental Developmental Index

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(MDI) of the BSID differed significantly across the three genotypes of *ABCB1* rs10276499 (2df F-test, p=0.045). With increasing cord MeHg, the MDI decreased (slope=−0.091, p=0.014) among children homozygous for the rare C-allele.

Conclusions: These findings support the possibility that child ABC genetics might influence prenatal MeHg exposure.

Introduction

Fish is an important source of protein for many people worldwide and it also contains other important nutrients, such as polyunsaturated fatty acids (PUFA), vitamins and minerals (Weichselbaum et al. 2013). However, fish also contains methylmercury (MeHg), which can cross the placenta and maternal exposure can therefore reach the fetus (Ask et al. 2002). MeHg is a neurotoxicant that can cause adverse effects on the central nervous system from industrial contamination (Sakamoto et al. 2018). The impact, if any, of fetal MeHg exposure from maternal fish consumption during pregnancy on children's neurological development is not clear. Some epidemiology studies have found negative associations between fetal MeHg exposure and neurodevelopment (Grandjean et al. 1997; Vejrup et al. 2016) whereas other studies have reported no association (Barbone et al. 2019; Daniels et al. 2004; Davidson et al. 1998; Llop et al. 2012; van Wijngaarden et al. 2017). One possible explanation for the discrepancy between studies may be genetic variation between populations.

Genetics may influence the degree of fetal MeHg exposure from the mother by affecting proteins involved in MeHg transport. Transport could also influence the uptake, metabolism, and excretion of MeHg as well as its movement across the placenta and blood brain barrier. One important pathway for MeHg metabolism and excretion involves the conjugation of glutathione (GSH) to MeHg in the liver followed by efflux across the cell membrane into the bile (Ballatori and Clarkson 1985). The ATP-binding cassette (ABC) transporter proteins are multidrug resistance-associated proteins (MRPs) involved in the transport of glutathione and various xenobiotics across the cell membrane. The ABC family includes, among others, ABCB1, ABCC1, and ABCC2 (also known as MDR1, MRP1 and MRP2). They are expressed in various tissues including the blood–brain barrier, placenta, liver, gut, and kidney where they participate in cellular export (Table 1).

Several studies, both in humans and animals, have demonstrated an association between ABC-transporters and MeHg exposure. However, the exact role of ABC transporters of MeHg regulation in humans and during pregnancy is not yet established. Based on the broad expression of ABC transporters in multiple tissue types, they could potentially be involved in several levels of MeHg transport and regulation during pregnancy including uptake via intestine and excretion via the liver in the mother, transfer over the placenta and blood-brain-barrier transfer in the child. In a previous study on this cohort, we reported that the maternal genotype for single nucleotide polymorphisms (SNPs) in *ABCC1*, *ABCC2* and *ABCB1* were associated with maternal hair MeHg concentrations in pregnant women (Engström et al. 2016). We also reported an association of rs11075290 T-alleles in *ABCC1* with improved neurological performance (measured as Mental Development Index and Psychomotor Development Index by the Bayley Scales of Infant Development) in the child

at 20 months (Engström et al. 2016). A study of two Mediterranean birth-cohorts reported that the associations between fish intake and cord blood MeHg were significantly different across of children's genotype of the same ABC transporters (Llop et al. 2014). These findings indicate that ABC transporter genetics may influence MeHg exposure, metabolism, dose, and effects in the child. Whereas these studies looked at either maternal or child genotypes, here we evaluate the association of both the mother's and child's genotype for SNPs in *ABCC1*, *ABCC2* and *ABCB1* with prenatal MeHg exposure and early child neurodevelopmental tests.

Materials and methods

Study population

The SCDS NC 2 is a prospective longitudinal epidemiology study investigating the association of prenatal MeHg exposure from maternal fish consumption during pregnancy, nutritional status, and genetics with children's developmental outcomes. We recruited the cohort between 2008 and 2011 and it consists of Seychellois mother-child pairs of mixed African, European and East Asian origin. We enrolled 1535 healthy mothers at their first antenatal visit (from 14 weeks of gestation) at eight health centers across the main Island of Mahé. Further information on inclusion and exclusion criteria and power calculations are described in Strain et al. 2015 (Strain et al. 2015). We collected maternal blood samples at 28 weeks gestation and maternal hair and cord blood at delivery. The Public Health Laboratory at the Ministry of Health processed the whole blood samples and then shipped them to the University of Rochester for Hg analysis and Lund University for genotyping.

The final cohort for the association of prenatal MeHg with SNPs was 946 mother-child pairs. Exclusions included 30 twin siblings and 559 children missing either child genetic data or cord blood Hg measurements. The final cohort for the analysis of SNPs and neurodevelopmental outcomes was 973. Exclusions included 30 twin siblings and 532 children who had no BSID-II or who met exclusion criteria related to neurodevelopmental testing.

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.

Hg measurements

Total Hg in cord whole blood was determined using Cold Vapor Atomic Absorption Spectroscopy (CVAAS) and a Laboratory Data Control Mercury Monitor Model #1235 as previously described (Magos and Clarkson, 1972; Cernichiari et al., 1995). The limit of detection (LOD) was 1.75 μg Hg/L. Total Hg was presumed to be primarily MeHg, as greater than 80% of total Hg in blood from fish consumers is reported to be MeHg (National Research Council, 2000; Sherlock et al., 1984; Phelps et al., 1980). Certified mercury standards (Fisher SM114–100 and Ricca Chemical Company AHG1KN-100) and certified reference material (Seronorm™, Sero) were utilized for internal quality control. For purposes

of external quality control, the laboratory participated in the Interlaboratory Comparison Program for blood sponsored by the Center of Toxicology of Quebec (INSPQ), Canada.

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 primary scores, the Mental Developmental Index (MDI) and the Psychomotor Developmental Index (PDI). Both scores are standardized with a mean =100 and an SD=15 and higher scores on each represent improved performance. 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).

Genotyping

In our previous study (Engström et al. 2016), we genotyped 15 maternal SNPs in ABC-transporters and examined their associations with MeHg exposures. Seven SNPs (rs2032582, rs10276499 and rs1202169 in *ABCB1*; rs11075290 and rs215088 in *ABCC1*; rs717620 in *ABCC2*) were significantly associated with maternal hair Hg (Engström et al. 2016), indicating their association with MeHg exposure. For the children, we selected those seven SNPs. Characteristics of the selected SNPs and the directions of the different genotype associations with maternal hair Hg in Engström et al. (2016) are presented in Table 1.

DNA for genotyping was extracted from cord blood using the Qiagen DNA Blood Mini kit (Qiagen, Hilden, Germany). Genotyping was performed by TaqMan real-time PCR on the ABI 7900HT Fast Real Time PCR System (Applied Biosystems, Thermo Fisher, Waltham, USA), using manufacturer's recommended standard conditions and the following custom genotyping assays from Thermo Scientific: C__11711720C_30 and C__11711720D_40 (rs2032582), C__29490882_10 (rs10276499), C__2982705_20 (rs1202169), C__3188828_10 (rs212093), C__27065543_10 (rs215088) C__2814642_10 (rs717620). For rs11075290 in *ABCC1*, it was discovered that an adjacent SNP, rs113499404, was situated within the probe sequence of the assay C_31910352 (Thermo Scientific) that was used for genotyping of NC2 mothers in Engström et al. (2016). Rs113499404 has a MAF <1% in all populations except Africans for which the average MAF is 5% (Ensemble Genome Browser: http://www.ensembl.org). Therefore, a new assay excluding rs113499404 was designed for rs11075290 and used in this study to generate genotype data for both mothers and children. *ABCB1* rs2032582 is tri-allelic and was genotyped by two different TaqMan assays to capture all three alleles. In the analyses of rs2032582, only subjects with the more frequent G and T alleles are included.

For quality control of genotyping data, >5% of samples were re-analyzed for all SNPs in a separate round of experiments with a 100% agreement between duplicates. Data quality was also assessed by evaluating Hardy-Weinberg Equilibrium (HWE) using the conventional Chi-Square test. All SNPs were in HWE except for rs212093 which showed a x^2 value of 7.26 (should be less than 3.8) and the rs212093 genotyping data was therefore excluded from statistical analyses.

Statistical analyses

Linear regression was used for analysis to estimate the association of each of the six children's SNPs with cord blood MeHg. We also re-ran these models adjusting for maternal genotypes for each of the SNPs. Linear regression was also used to estimate the association of each SNP separately with BSID-II scores in models with only child genotype and with both child and maternal genotype. In the BSID-II models, we also adjusted for child sex, maternal age at delivery, presence of two parents in the household, Hollingshead socioeconomic score, and child age at testing. Statistical significance was assigned to two-sided p-values less than 0.05.

Similar to other studies on gene-metal interactions (Broberg et al. 2019; Tian et al. 2013), we also investigated whether polymorphisms in ABC transporter genes could influence the relationship between cord blood MeHg concentrations and neurodevelopment, by including the interaction between SNPs (coded with 3 levels for three possible genotypes, see Table 3 for the genotypes for each SNP) and cord blood MeHg in models for BSID-II scores. In these models, we considered the interaction significant when the 2 degree of freedom interaction p-value was 0.05 or less. In this case, the cord blood MeHg association with the BSID-II scores was reported separately for each genotype. Statistical analyses were undertaken using R (version 3.3.2; The R Foundation for Statistical Computing).

For the analyses described, we performed post-hoc power calculations. A small effect size for a linear model is f^2=0.02 (Cohen 1988). With the smallest sample size for each analysis type (see Tables 2–3 and S1 for sample sizes), our power to detect an effect size of at least 0.02 is 97.2% for testing gene effects on MeHg, 96.5% for testing gene effects on BSID-II scores, and 95.0% for testing gene-MeHg interactions on BSID-II scores.

Results

Minor allele frequencies of SNPs in NC2 mothers and children are presented in Table 1 and other summary statistics for study subjects are presented in Table 2. SNP allele frequencies were consistent between mothers and children and also with publicly available genetic data of related populations (acquired from the Ensemble Genome Browser (https://www.ensembl.org) (Table 1).

Associations of child and maternal ABC-genetics with prenatal MeHg

In Table 3, we present results from analyses of associations of fetal MeHg with genotypes for each SNP. One model includes only child genotype and the other both child and maternal genotype. Only *ABCC1* rs11075290 showed a significant association with cord MeHg. The p-value for the association with adjustment for maternal genotype was p=0.0008 and without adjustment was p=0.0001. The pattern showed decreasing cord blood MeHg levels with the number of rare alleles: children homozygous for the rare allele (TT) had on average 29.99 μg/L MeHg in cord blood while heterozygotes (CT) had 33.32 μg/L and the common allele homozygotes (CC) had 38.06 μg/L.

Associations of child ABC genetics with early neurodevelopmental outcomes and interaction with exposure

There were no significant associations of the child ABC-genotype with any neurodevelopmental test outcomes (MDI or PDI) at 20 months (Supplemental Table 1). The associations between the maternal ABC-genotype and test outcomes were also not significant. However, the changes in both MDI and PDI between different maternal ABCC1 rs11075290 genotypes are similar in magnitude to those previously reported in Engström et al. (2016), but were not statistically significant after adjusting for the child genotype.

Interactions of prenatal MeHg exposure with child ABC genetics and early neurodevelopmental testing

We observed a statistically significant interaction (p=0.045) only for child *ABCB1* rs10276499 genotype association with cord blood MeHg concentrations on the MDI (Fig. 1). There was no association between cord blood MeHg and MDI among participants with TT (slope=0.024, CI=(−0.03,0.07), p=0.353) or CT (slope=0.0014, CI=(−0.05,0.05), p=0.955). However, participants with the rare homozygous allele CC the MDI scores declined with increasing cord MeHg (slope=−0.091, CI=(−0.17,−0.01), p=0.045) (Fig. 1). The interaction remained statistically significant (p=0.022) when maternal genotype was included in the models. No other interaction models were significant.

Discussion

In this study, one child SNP out of six, showed a significant association with cord MeHg. For *ABCC1* rs11075290, having more of the rare T-alleles was associated with lower concentrations of cord MeHg, indicating that this gene may affect MeHg exposure in the unborn child. This is in concordance with a study of European (Italian, Greek and Spanish) mother-child cohorts where children carrying the rs11075290 T-allele showed significantly lower cord MeHg in the Italian and Spanish cohorts (Llop et al. 2014, of note, no maternal genetics for the cohort was evaluated in that study).

We did not find any association between maternal rs11075290 and cord blood MeHg. This is consistent with the predominant placental localization of ABCC1 which is on the abluminal surface composed of fetal endothelial cells and to a lesser extent at the basal (fetal-facing) membrane of the syncytiotrophoblast layer (Nagashige et al. 2003; St-Pierre et al. 2000) and thus coded by the fetal genome. The primary role of ABCC1 in the fetus appears to be the clearance of substances from the fetal blood (St-Pierre et al. 2000). Rs11075290 is a non-coding SNP situated in the first intron of *ABCC1* in a region which displays signatures of regulatory potential including cis-regulatory elements and histone acetylation (UCSC Genome Browser, Accessed June 2021). Rs11075290 is categorized as an expression quantitative trait locus (eQTL) and the T-allele is associated with increased ABCC1 expression in multiple tissues (the GTEx Portal, Accessed, 10 Dec 2019) which may be caused by the disruption of a CpG site that could alter the methylation profile. A higher expression of ABCC1 (from the T-allele) would presumably result in increased fetal blood clearance which is concordant with lower cord blood MeHg as observed in our study.

Despite the strong association between child rs11075290 and cord MeHg, we did not observe any association between child rs11075290 and neurodevelopmental outcomes. Engström et al. (2016) reported that maternal rs11075290 T-allele was significantly associated with higher scores on both the MDI and PDI scores from the BSID. When adding the child rs11075290 genotype into the model, the effect estimates for the maternal genotype remained similar in magnitude, but the association became non-significant. One possible explanation for this finding might be that the fetal ABCC1 expression is low in early gestation and that the exposure in the fetus at this stage is dominated by the maternal MeHg exposure. If true it would be unfortunate since the fetal developing brain is particularly sensitive to MeHg. In support of this possible explanation, ABCC1 expression in the placenta, which is mainly a tissue of fetal origin, has been shown to increase from 1st trimester to 3rd trimester (Pascolo et al. 2003).

We also observed an interaction between *ABCB1* rs10276499 and cord MeHg on the MDI indicating a potential protective influence of the T-allele. ABCB1 is expressed in fetal brain from an early stage and is likely protecting the developing brain against xenobiotics from the mother (Han et al. 2018). The fact that this SNP only is associated with neurodevelopment in an interaction model, may hypothetically reflect that the influence of this SNP on MeHg toxicity is dependent on the MeHg concentration.

The strength of this study is the large, well-characterized cohort of mother child pairs with a MeHg exposure resulting from a high fish-intake. Additionally, the genetic data for ABC SNPs in both mothers and children allowed us to examine the contribution of both child and mother genetic backgrounds to MeHg exposure and association with neurodevelopment and also test for interactions. In the final analysis examining interactions between prenatal MeHg exposure with ABC genetics on early neurodevelopmental outcomes, we compare subjects with varying MeHg exposures including the whole observed range of low and high exposures. This method estimates effects of prenatal MeHg on outcomes within each genotype. A limitation is the number of subjects with genetic data may still be too low to detect subtle changes or be reliable for evaluation of interactions. We did not find many associations between ABC SNPs, MeHg concentrations, and neurodevelopment. This may reflect that the ABC transporters are multitasking proteins (Cole 2014; Tomas & Tampé 2020) and there may be other factors that we did not account for, e.g. in the diet, that influence the ABC expression, and in turn influence associations between MeHg concentrations and neurodevelopment. It is therefore necessary to investigate the role of ABC transporters in populations with other genetic and environmental backgrounds.

In conclusion, our study adds support to the hypothesis that the child's ABC genetics may influence prenatal MeHg exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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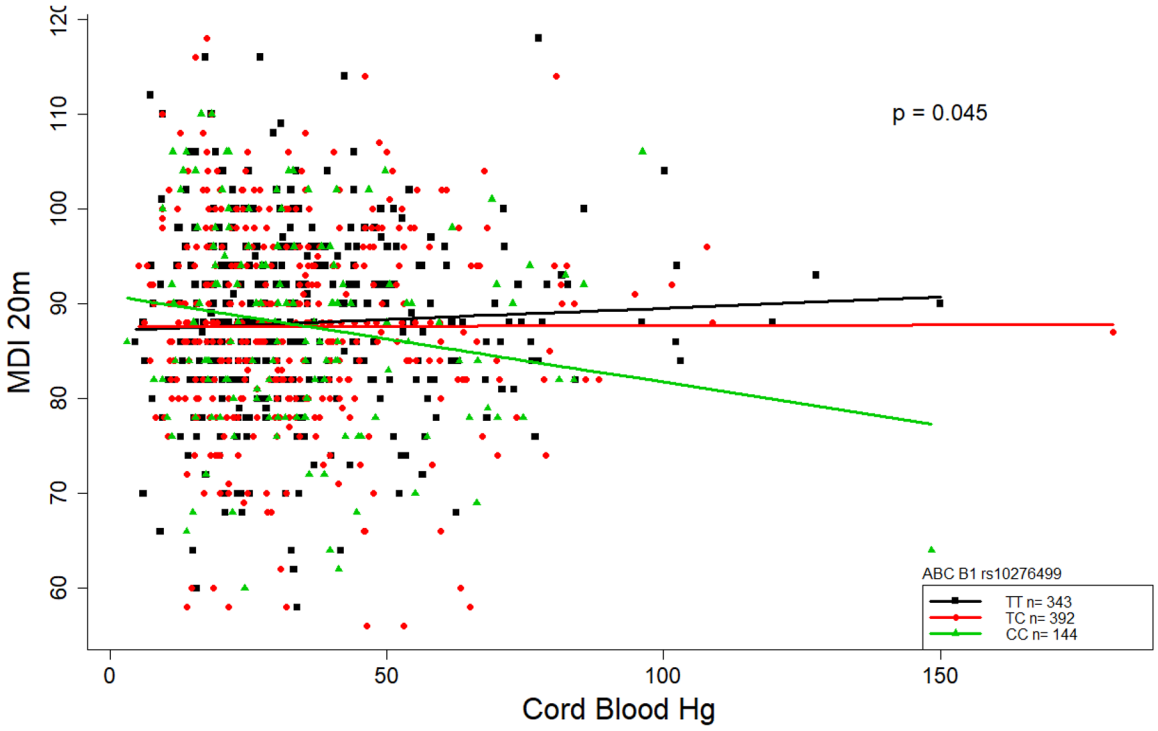

Figure 1:

The relationship between cord blood MeHg levels and scores on the mental development index (MDI) from the interaction model. The *ABCB1* rs10276499 of children is significantly modifying the association between cord blood MeHg and MDI (2df test for interaction, p=0.045). There was no association between cord blood MeHg and MDI among those with TT (slope=0.024, CI=(−0.03,0.07), p=0.353) or CT (slope=0.0014, CI=(−0.05,0.05), p=0.955), whereas for the rare allele homozygotes (CC), MDI scores declined with increasing cord blood MeHg (slope=−0.091, CI=(−0.17,−0.01), p=0.045). The results of the interaction models (including this one) are not presented in any table; this is the only significant interaction between child SNPs and cord blood MeHg out of the six SNPs and two outcomes considered.

Table 1.

Summary of current knowledge and distribution in different populations for the six SNPs evaluated in the study.

Gene/ protein	Expression pattern	Gene function	SNP	SNP type/ predicted effect[a]	Alleles major/ minor	Association of minor allele in NC2 mothers (Engstrom et al. 2016)	Minor allele frequencies				
							NC2 children	NC2 mothers	AFR[a]	EUR[a]	SAS
ABCC1/ MRP1	High expression in lung, spleen, testis, kidney, thyroid, bladder, adrenal gland (Yin and Zhang 2011) and basal membrane of placental syncytiotrophoblast (Nagashige et al. 2003). Also expressed in blood-brain, blood-testis and blood-cerebrospinal fluid (CSF) barriers (Yin and Zhang 2011)	Cellular efflux of a wide range of substrates including GSH conjugates. Location in basolateral membrane of polarized cells indicating transport towards the interstitial space rather than excretion (Yin and Zhang 2011). May be involved in transport both to and from fetal circulation over the placenta (Joshi et al. 2016).	rs11075290	Intronic/ regulatory	C/T	Higher hair MeHg; Higher (i.e. better) MDI and PDI scores	0.36	0.37	0.30	0.57	0.40
			rs215088	Intronic/ regulatory	G/A	Lower hair Hg	0.40	0.41	0.52	0.28	0.20
ABCC2/ MRP2	Liver, kidney, intestine, blood-brain barrier (Dombrowski et al. 2001) and maternal apical membrane of placenta (Meyer zu Schwabedissen et al. 2005)	Cellular efflux and biliary excretion of endogenous and exogenous waste products, mostly as conjugates (Jemnitz et al. 2010). Limitation of xenobiotic absorption and xenobiotic clearance in the intestine (Tocchetti et al. 2016). Placental transfer toward maternal blood (Vahakangas and Myllynen 2009).	rs717620	5′ UTR/ regulatory	C/T	Higher hair MeHg	0.10	0.10	0.03	0.21	0.10
ABCB1/ MDR1, P-glyco-protein	Expressed in kidney, pancreas, liver, jejunum, colon, adrenal gland, (Thiebaut et al. 1987), endothelial cells of the blood–brain barrier (Tatsuta et al. 1992) and placenta (Sun et al. 2006).	Transmembrane efflux transporter with a broad range of endogenous and xenobiotic substrates. Involved in excretion of possibly toxic compounds via bile and kidney, preventing their entry over blood-brain barrier and placenta (Han et al. 2018; Wolking et al. 2015).	rs2032582	Non-synonymous missense/ altered protein function	G(Ala)/ T(Ser)/ A(Thr)	Higher hair MeHg	0.19 (T)	0.18 (T)	0.02	0.41	0.59
			rs10276499	Intronic/ regulatory	T/C	Lower hair MeHg	0.38	0.38	0.39	0.06	0.06
			rs1202169	Intronic	T/C	Higher hair MeHg	0.34	0.34	0.19	0.41	0.60

[a]SNP information and allele frequencies were obtained from Ensemble Genome Browser (www.ensembl.org) accessed June 2021.

Table 2.

Summary statistics for the exposure and covariate variables from NC2 mothers and children that were included in the study.

Variable	n	mean	min, max	5, 95 percentile
Cord blood MeHg (μg/L)	946	35	2, 181	11, 76
Child test age (months)	973	21	16, 33	18,23
Maternal age at delivery (years)	973	27	16, 45	18, 39
Hollingshead SES	973	32	11, 63	17,50
Family status (% with 2 parents in household)	973	73%		
Percent girls	973	50%		

Table 3.

Mean cord blood (prenatal) MeHg concentrations by child and maternal genotypes of ABC transporter SNP. For each SNP, the p-value for the test comparing the means in the three groups is reported.

			Model with only child genotype				Model with both child and maternal genotype							
			Child genotype				Child genotype (adjusted for maternal)				Maternal genotype (adjusted for child)			
Gene	**SNP**	**Geno-type**	**n**	**Mean MeHg (μg/L)**	**95% CI**	**p[a]**	**n**	**Mean MeHg (μg/L)**	**95% CI**	**p[a]**	**n**	**Mean MeHg (μg/L)**	**95% CI**	**p[a]**
ABCB1	rs2032582	GG	575	35.05	(33.31,36.80)	0.87	537	35.16	(31.88,38.43)	0.94	554	34.05	(30.90,37.19)	0.15
		GT	274	35.26	(32.73,37.79)		258	34.14	(30.96,37.32)		249	34.14	(30.96,37.32)	
		TT	43	33.40	(27.02,39.79)		41	31.88	(24.97,38.78)		33	41.94	(34.23,49.66)	
	rs10276499	TT	374	35.54	(33.40,37.68)	0.57	362	35.86	(32.72,39.00)	0.64	358	33.23	(30.07,36.40)	0.72
		TC	421	34.03	(32.02,36.05)		408	34.15	(31.69,36.62)		413	34.15	(31.69,36.62)	
		CC	148	35.35	(31.95,38.75)		140	35.38	(31.49,39.26)		139	35.22	(31.32,39.12)	
	rs1202169	TT	415	35.47	(33.44,37.50)	0.40	405	34.28	(31.50,37.07)	0.17	396	34.42	(31.58,37.25)	0.36
		TC	415	33.82	(31.79,35.85)		395	32.31	(29.82,34.79)		419	32.31	(29.82,34.79)	
		CC	116	36.19	(32.36,40.03)		112	36.14	(31.88,40.39)		97	33.90	(29.32,38.49)	
ABCC1	rs11075290	CC	389	38.06	(35.98,40.14)	**<0.01**	378	36.96	(34.11,39.81)	**<0.01**	371	33.46	(30.57,36.35)	0.32
		CT	424	**33.32**	**(31.33,35.31)**		404	**32.09**	**(29.57,34.60)**		407	32.09	(29.57,34.60)	
		TT	128	**29.99**	**(26.37,33.61)**		123	**28.77**	**(24.81,32.74)**		127	35.13	(31.23,39.03)	
	rs215088	GG	336	35.56	(33.3,37.82)	0.39	326	35.76	(32.75,38.78)	0.34	328	33.65	(30.65,36.65)	0.99
		GA	460	33.87	(31.94,35.8)		440	33.74	(31.30,36.17)		423	33.74	(31.30,36.17)	
		AA	144	36.09	(32.65,39.54)		142	35.91	(32.00,39.82)		157	33.87	(30.16,37.57)	
ABCC2	rs717620	CC	760	34.63	(33.12,36.13)	0.75	732	34.72	(30.87,38.57)	0.84	715	34.85	(30.78,38.92)	0.68
		CT	170	35.62	(32.44,38.80)		166	34.88	(31.21,38.55)		186	34.88	(31.21,38.55)	
		TT	12	37.98	(26.02,49.93)		12	37.98	(26.02,49.93)		9	41.23	(27.42,55.04)	

[a]The p-values are comparing the means between the three genotypes. The means in bold are significantly different from the reference homozygote (listed first).