



Polymorphisms in ATP-binding cassette transporters associated with maternal methylmercury disposition and infant neurodevelopment in mother-infant pairs in the Seychelles Child Development Study

Engstrom, K., Love, T. M., Watson, G. E., Zareba, G., Yeates, A. J., Wahlberg, K., Alhamdow, A., Thurston, S. W., Mulhern, M. S., McSorley, E. M., Strain, J.J., Davidson, P. W., Shamlaye, C. F., Myers, G. J., Rand, M. D., van Wijngaarden, E., & Broberg, K. (2016). Polymorphisms in ATP-binding cassette transporters associated with maternal methylmercury disposition and infant neurodevelopment in mother-infant pairs in the Seychelles Child Development Study. *Environment International*, 94, 224-229. <https://doi.org/10.1016/j.envint.2016.05.027>

[Link to publication record in Ulster University Research Portal](#)

Published in:
Environment International

Publication Status:
Published (in print/issue): 30/09/2016

DOI:
[10.1016/j.envint.2016.05.027](https://doi.org/10.1016/j.envint.2016.05.027)

Document Version
Author Accepted version

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1 **Polymorphisms in ATP-binding cassette transporters associated with maternal**
2 **methylmercury disposition and infant neurodevelopment in mother-infant pairs in the**
3 **Seychelles Child Development Study.**

4

5 Karin Engström^{a,b}, Tanzy M Love^c, Gene E Watson^c, Grazyna Zareba^c, Alison Yeates^d, Karin
6 Wahlberg^a, Ayman Alhamdow^b, Sally W Thurston^c, Maria Mulhern^d, Emeir M McSorley^d, JJ
7 Strain^d, Philip W Davidson^c, Conrad F Shamlaye^e, GJ Myers^c, Matthew D Rand^c, Edwin van
8 Wijngaarden^c and Karin Broberg^{a,b*}

9

10 ^a Department of Laboratory Medicine, Division of Occupational and Environmental Medicine,
11 Lund University, 22185 Lund, Sweden

12 ^b Institute of Environmental Medicine (IMM), C6, Metals and health, Box 210, 171 77
13 Stockholm, Sweden

14 ^c University of Rochester Medical Center, School of Medicine and Dentistry, 601 Elmwood
15 Ave, Box 671, Rochester, NY 14642, USA

16 ^d the Northern Ireland Centre for Food and Health (NICHE), School of Biomedical Sciences,
17 University of Ulster, Coleraine campus, Cromore Road, Coleraine, Co. Londonderry, BT52
18 1SA, Northern Ireland

19 ^e the Child Development Centre, Ministry of Health, Mahé, Republic of Seychelles

20

21 *To whom correspondence should be addressed: Professor Karin Broberg, Institute of
22 Environmental Medicine (IMM), C6, Metals and health, Box 210 171 77 Stockholm, Sweden
23 E-mail: karin.broberg@ki.se. Tel: +46737823750. Fax: +468336981.

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25 **Keywords:** ABC transporter, MRP1, MRP2, MDR1, p-glycoprotein, neurodevelopment

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ABC, ATP-binding cassette; ABCB1, ATP-Binding Cassette, Sub-Family B (MDR/TAP), Member 1; ABCC1, ATP-Binding Cassette, Sub-Family C (CFTR/MRP), Member 1; ABCC2, ATP-Binding Cassette, Sub-Family C (CFTR/MRP), Member 2; BSID-II, Bayley scales of infant development II; MDI; mental development index; MDR1, multidrug resistance protein 1; MeHg, methylmercury; MRP1, Multidrug resistance-associated protein 1; MRP2, Multidrug resistance-associated protein 2; NC2, nutrition cohort 2; PDI, psychomotor development index; SCDS, Seychelles child development study; SNP, single nucleotide polymorphism

31 **Abstract**

32 Background: ATP-binding cassette (ABC) transporters have been associated with
33 methylmercury (MeHg) toxicity in experimental animal models.

34 Aims: To evaluate the association of single nucleotide polymorphisms (SNPs) in maternal
35 ABC transporter genes with 1) maternal hair MeHg concentrations during pregnancy and 2)
36 child neurodevelopmental outcomes.

37 Materials and methods: Nutrition Cohort 2 (NC2) is an observational mother-child cohort
38 recruited in the Republic of Seychelles from 2008-2011. Total mercury (Hg) was measured in
39 maternal hair growing during pregnancy as a biomarker for prenatal MeHg exposure
40 (N=1313) (mean 3.9 ppm). Infants completed developmental assessments by Bayley Scales of
41 Infant Development II (BSID-II) at 20 months of age (N=1331). Genotyping for fifteen SNPs
42 in *ABCC1*, *ABCC2* and *ABCB1* was performed for the mothers.

43 Results: Seven of fifteen ABC SNPs (*ABCC1* rs11075290, rs212093, and rs215088; *ABCC2*
44 rs717620; *ABCB1* rs10276499, rs1202169, and rs2032582) were associated with
45 concentrations of maternal hair Hg ($p < 0.001$ to 0.013). One SNP (*ABCC1* rs11075290) was
46 also significantly associated with neurodevelopment; children born to mothers with
47 rs11075290 CC genotype (mean hair Hg 3.6 ppm) scored on average 2 points lower on the
48 Mental Development Index (MDI) and 3 points lower on the Psychomotor Development
49 Index (PDI) than children born to mothers with TT genotype (mean hair Hg 4.7 ppm) while
50 children with the CT genotype (mean hair Hg 4.0 ppm) had intermediate BSID scores.

51 Discussion: Genetic variation in ABC transporter genes was associated with maternal hair Hg
52 concentrations. The implications for MeHg dose in the developing child and
53 neurodevelopmental outcomes need to be further investigated.

54

55 **1. Introduction**

56 Fish is a primary source of protein for more than four billion people worldwide (FAO/WHO
57 2011). Methylmercury (MeHg) is a well-documented neurotoxicant that is present in all fish
58 to a varying extent. At adequate dosages, MeHg poses a risk for the developing fetus, as it is
59 known to readily cross the placenta and the blood-brain barrier (WHO 2007). However, the
60 results concerning consequences of methylmercury exposure naturally found in fish on child
61 neurodevelopment has been contradictory. While studies in New Zealand, Faroe Islands, and
62 the United States have reported adverse developmental influences associated with prenatal
63 MeHg exposure (Crump et al. 1998; Grandjean et al. 1997; Oken et al. 2005; Sagiv et al.
64 2012), no overall association between prenatal MeHg exposure and developmental outcomes

65 were observed in large studies in the Seychelles, United Kingdom and Spain (Daniels et al.
66 2004, Davidson et al. 1998; Llop et al. 2012, Myers et al. 2003, Strain et al. 2015). These
67 results reveal that the association between prenatal MeHg exposure from maternal fish
68 consumption and child developmental outcomes is far more complex than previously thought
69 and inconsistencies among study findings may be due to variability in concomitant dietary
70 exposures or genetic factors influencing MeHg toxicity. Several factors influence the
71 concentration of MeHg experienced by the fetus, such as the type and the amount of fish
72 consumed; however, the contribution of the genetic background has rarely been studied.
73 Sequence variation in maternal genes responsible for the toxicokinetics of MeHg is likely to
74 result in differences in MeHg concentrations, and in turn differences in neurotoxicity,
75 between infants of mothers with a similar fish intake. Yet, very little is known about gene –
76 MeHg interactions on child neurodevelopment (Llop et al. 2015).

77 Animal studies provide evidence that transporter proteins can affect MeHg toxicokinetics.
78 The ATP-binding cassette (ABC) transporters Multidrug resistance-associated protein 1
79 (MRP1, gene name ATP-Binding Cassette, Sub-Family C (CFTR/MRP), Member 1
80 (*ABCC1*)), Multidrug resistance-associated protein 1 (MRP2, gene name ATP-Binding
81 Cassette, Sub-Family C (CFTR/MRP), Member 2 (*ABCC2*)) and Multidrug resistance protein
82 1 (MDR1, gene name ATP-Binding Cassette, Sub-Family B (MDR/TAP), Member MDR1
83 (*ABCB1*)) are the best-characterized ABC transporters. *ABCC1* and *ABCC2* have been
84 associated with MeHg accumulation and toxicity in fruit flies and mice (Bridges and Zalups
85 2005; Prince et al. 2014; Toyama et al. 2011). The role of *ABCB1* in MeHg transport and
86 toxicity is not yet known. These three ABC transporter genes are highly conserved across
87 species and are abundant in tissues where MeHg transport is most critical (e.g. blood-brain-
88 barrier, liver, gut and the placenta). The role of ABC transporters in MeHg transport suggests
89 that single nucleotide polymorphisms (SNPs) in ABC transporters may influence MeHg body
90 burden in both mother and fetus during pregnancy. Indeed, in a study based on two European
91 birth cohorts, it was found that child SNPs in *ABCC1*, *ABCC2*, and *ABCB1* modified the
92 relationship between maternal fish consumption and cord blood Hg concentrations (Llop et al.
93 2014). However, it is not known whether ABC SNPs influence the concentration of maternal
94 hair Hg, a biomarker often used as a proxy for prenatal exposure to MeHg (Davidson et al.
95 2008). The potential influence of ABC genotype on MeHg body burden in pregnant mothers
96 or their children may mediate adverse associations with developmental outcomes; however,
97 few studies have investigated the association between ABC SNPs and child
98 neurodevelopment directly. The current study comprises a mother-child cohort from the

99 Seychelles Child Development Study (SCDS), characterized by high fish intake and hair Hg
100 concentrations which are substantially higher than in many other populations. Here, we
101 characterize the associations of maternal SNPs in ABC transporters with hair Hg
102 concentrations during pregnancy and with child neurodevelopmental outcomes.

103

104

105 **2. Methods**

106 *2.1 Study population*

107 The SCDS is a longitudinal observational study conducted in the Republic of Seychelles, an
108 archipelago in the Indian Ocean. The population resides mainly on the island of Mahé and is
109 of mixed African, European, and East Asian origin. The overall aim of the SCDS is to
110 investigate the effects of MeHg exposure during pregnancy on child developmental outcomes.
111 Healthy mothers were recruited to Nutrition Cohort 2 (NC2) during their first antenatal visit
112 (from 14 weeks of gestation) at eight health centres across Mahé. Mothers were enrolled from
113 2008 until 2011 when the target number of 1500 mothers had consented (Strain et al. 2015).
114 Mothers with double pregnancies were only counted once in analyses of developmental
115 outcomes (one of each sibling pair was removed randomly). One hair Hg value was an outlier
116 (194.3 ppm, the next highest observed was 31.66 ppm); the Hg was found to be primarily
117 inorganic and this value was therefore excluded from the Hg analyses. After exclusions owing
118 to missing data (Strain et al. 2015), there were 1313 mothers who were included in the
119 analyses of MeHg toxicokinetics and 1331 mother-child pairs were included in analyses of
120 developmental outcomes. Further information on inclusion criteria and power calculations has
121 previously been described (Strain et al. 2015). The study was reviewed and approved by the
122 Seychelles Ethics Board, the Research Subjects Review Board at the University of Rochester,
123 and the Regional Ethics Committee at Lund University, Sweden.

124

125 *2.2 Hair Hg analyses*

126 Hair samples were cut at delivery and the longest available segment of maternal hair growing
127 during gestation was analysed assuming a hair growth rate of 1.1 cm/month. Total mercury in
128 maternal hair during gestation is a known biomarker for prenatal MeHg exposure and was
129 measured by cold-vapor Atomic-Absorption-Spectrometry as previously described
130 (Cernichiari et al. 1995) and reported in parts per million (ppm).

131

132 *2.3 Developmental assessment*

133 When infants were aged approximately 20 months, they completed developmental testing
134 with a Creole version of the Bayley Scales of Infant Development (BSID-II), which has been
135 successfully used in a previous Seychellois cohort (Davidson et al. 2008) as well as in the
136 current NC2 cohort (Strain et al. 2015). Testing was conducted by specially trained nurses at
137 the Child Development Centre, Victoria, Mahé. All study forms were shipped to the
138 University of Rochester, where data were double-entered and the Mental Development Index
139 (MDI) and Psychomotor Development Index (PDI) endpoints were scaled according to the
140 child's age at testing. Test reliability for the BSID-II was determined as previously described
141 (Strain et al. 2015).

142

143 *2.4 DNA extraction and genotyping*

144 DNA was extracted from maternal blood using the Qiagen DNA Blood Mini kit (Qiagen,
145 Hilden, Germany). SNPs were genotyped by the iPLEX® Gold assay on the MassARRAY
146 platform (Sequenom™, San Diego, USA) and by TaqMan allelic discrimination assay on an
147 ABI 7900 instrument (Applied Biosystems, Foster City, CA, USA), according to the
148 manufacturer's instructions. We characterized the variation in the ABC genes by choosing
149 TagSNPs that capture as much of the genetic variation within a gene as possible due to
150 linkage disequilibrium with other (not genotyped) SNPs. TagSNPs were selected according to
151 HapMap data (Thorisson et al. 2005) for YRI (Yoruba in Ibadan, Nigeria) as a proxy for
152 African populations, since earlier screenings of this population have shown that the allele
153 frequencies are more similar to YRI than the other Hapmap populations (Yeates et al. 2015).
154 In total, we genotyped 15 SNPs (description of all SNPs is found in Table 1) including the
155 three SNPs that previously had been linked to cord blood Hg in Mediterranean cohorts:
156 *ABCC1* rs11075290, *ABCC2* rs2273697, and *ABCB1* rs2032582 (Llop et al. 2014). *ABCB1*
157 rs2032582 is tri-allelic and was genotyped by two different TaqMan assays to capture all
158 three alleles. Five percent of the samples were re-analyzed for quality control purposes with
159 perfect agreement between original and repeat genotyping runs for all SNPs.

160

161 *2.5 Statistical analyses*

162 Deviations from Hardy-Weinberg equilibrium were tested using chi-square analysis. Linkage
163 disequilibrium was evaluated using Haploview (Barrett et al. 2005). Tests for associations
164 between outcomes and SNPs were carried out from *a priori* analysis plans and all associations
165 were evaluated using two-sided tests of significance at the $\alpha=0.05$ level. For the tri-allelic
166 *ABCB1* rs2032582 we excluded genotypes with the A-allele due to allele frequency <5%

167 (there were 60 subjects with GA, AT and AA genotype removed from the analysis of genetic
168 association with maternal hair Hg concentrations and 61 from the analysis of genetic
169 associations with child BSID-II); after this removal there were three SNP levels. All statistical
170 analyses were undertaken using R (version 3.0.2; The R Foundation for Statistical
171 Computing).

172 Simple linear regression with no covariate adjustment (assuming that fish consumption
173 patterns and other determinants of MeHg exposure are similar for mothers with different
174 SNPs) was used to estimate the association of each of the ABC SNPs with maternal hair Hg
175 concentrations. We used a 2 degree of freedom test to evaluate differences in hair Hg across
176 the three levels of each SNP. Multiple linear regression was also used to estimate the
177 association of ABC SNPs with BSID-II scores and these were adjusted for covariates
178 previously chosen to cover the most important determinants of neurocognitive development in
179 children (Strain et al. 2015).

180 **Table 1.** Single nucleotide polymorphisms (SNPs) included in the NC2 study. All genotyped individuals included in either the association
 181 analyses with hair Hg or with BSID analyses are included (n=1410-1416 depending on the SNP).

Gene	SNP ^a	SNP type ^b	Functional effect ^c	Allele freq. NC2	Allele freq. YRI ^d	Allele freq. CEU ^e
<i>ABCC1</i>	rs215088	Intronic A/G	regulatory variant - enhancer	0.41 (A)	0.59	0.29
<i>ABCC1</i>	rs1292798	Intronic G/T	regulatory variant - promotor flanking	0.05 (T)	0.10	0.08
<i>ABCC1</i>	rs1107529	Intronic C/T	regulatory variant - promotor flanking	0.37 (T)	0.30	0.55
<i>ABCC1</i>	rs246241	Intronic G/T	regulatory variant - promotor flanking	0.24 (T)	0.26	0.11
<i>ABCC1</i>	rs212093	5' near gene G/A	no predicted change of function	0.41 (A)	0.38	0.57
<i>ABCC2</i>	rs717620	5' untranslated region C/T	regulatory variant – promotor	0.10 (T)	0.03	0.21
<i>ABCC2</i>	rs2756103	Intronic A/C	regulatory variant - promotor flanking	0.29 (C)	0.23	0.42
<i>ABCC2</i>	rs7393105	Intronic A/C	no predicted change of function	0.36 (C)	0.40	0.42
<i>ABCC2</i>	rs2273697	Non-synonymous,	predicted tolerated	0.20 (A)	0.25	0.20
<i>ABCB1</i>	rs2032582	Non-synonymous, Ala(G)/Ser(T) /Thr(A)	Ser ->Ala predicted tolerated Ser ->Thr predicted deleterious	0.79(G)/ 0.18(T)/ 0.02(A)	100 (G)	0.56/0.43/ 0.01
<i>ABCB1</i>	rs2235035	Intronic G/A	no predicted change of function	0.21 (A)	0.21	0.31
<i>ABCB1</i>	rs1027458	Intronic G/A	no predicted change of function	0.16 (A)	0.20	0.13
<i>ABCB1</i>	rs1202169	Intronic T/C	no predicted change of function	0.34 (C)	0.19	0.42
<i>ABCB1</i>	rs1202171	Intronic T/A	no predicted change of function	0.25 (T)	0.36	0.37
<i>ABCB1</i>	rs1027649	Intronic C/T	no predicted change of function	0.38 (C)	0.35	0.06

182 ^a SNPs are ordered according to their position in the gene at 5' to 3' direction, according to Human genome assembly 38 (GRCh38). *ABCB1* is
 183 situated on chromosome 7, *ABCC1* on chromosome 16, and *ABCC2* on chromosome 10.

184 ^b Ancestral allele is denoted first.

185 ^c According to www.ensembl.org. Amino acid exchange predictions are from “Sorting Intolerant from tolerant” (SIFT) and from PolyPhen-2
 186 (Polymorphism Phenotyping v2) at Ensembl.

187 ^d Data from Hapmap (www.hapmap.org) for an African population (YRI, Yoruba in Ibadan, Nigeria).

188 ^e Data from Hapmap (www.hapmap.org) for a European population (CEU, Utah Residents with Northern and Western European ancestry).

189 The covariates included child sex, maternal age at delivery, presence of two parents in the
 190 household, Hollingshead socioeconomic score, and child age at testing. The models for the
 191 BSID MDI and PDI did not include maternal hair Hg because as a potential mediator it would
 192 affect our ability to estimate the direct association between SNPs and BSID scores. Because
 193 there were many missing values for maternal hair Hg, our sample sizes for these analyses
 194 were considerably larger than those reported in Strain et al. (2015).

195

196 3. Results

197 Summary statistics for the cohort are shown in Table 2. All SNPs were in Hardy-Weinberg
 198 equilibrium. *ABCC2* (rs7393105 and rs2756103) were in linkage disequilibrium ($R^2 = 0.72$),
 199 while all other pairwise combinations of SNPs had a R^2 lower than 0.3.

200 **Table 2.** Summary statistics for the Nutrition Cohort 2 in the Seychelles Child Development
 201 Study.

Variable	N	Mean	SD
Maternal hair Hg (ppm)	1313	3.94	3.47
Fish meals per week	1243	8.58	4.63
Birthweight (g)	1305	3172	498
Gestational age (weeks)	1262	38.96	1.61
BSID score MDI	1327	87.63	10.74
BSID score PDI	1325	96.69	10.59
% Girls	1331	48%	
% Two parents in the household	1331	73%	
Maternal age (years)	1331	27.06	6.29
Hollingshead SES	1331	32.04	10.35

202 *Abbreviations: N= Number of individuals, SD = Standard deviation, Hg = mercury,*

203

204 SNPs in all three ABC genes were significantly associated ($p < 0.050$) with maternal hair Hg
 205 (Table 3). None of these SNPs were in linkage disequilibrium. Three SNPs in *ABCC1*
 206 (rs11075290, rs212093, and rs215088), one SNP in *ABCC2* (rs717620), and three SNPs in
 207 *ABCB1* (rs10276499, rs1202169, and rs2032582) were associated with hair Hg
 208 concentrations, and all but two (*ABCC1* rs212093 and rs215088) showed an allele-
 209 concentration effect. For example, the mean hair Hg among CC carriers of rs11075290 was 1
 210 ppm (~20%) lower than the mean hair Hg among TT carriers. Using Hapmap data, we
 211 compared this population with those of African and European ancestry. The alleles associated
 212 with higher concentrations of Hg in hair in this study (Table 3) showed in general a higher

213 allele frequency in Hapmap individuals with European ancestry, but a lower allele frequency
 214 in Hapmap individuals with African ancestry, compared with in NC2 (Table 1).

215

216 **Table 3.** Associations between maternal *ABC* genotypes, maternal hair Hg, and the BSID
 217 MDI and PDI ^a.

Gene/SNP	Hair Hg			MDI				PDI					
	N	Mean	P	N	Mean	β	P	N	Mean	β	P		
<i>ABCC1 (MRP1)</i>													
rs215088	GG	470	4.3	480	87.6	-	0.34	479	96.8	-	0.42		
	GA	622	3.7		621	88.0			0.42	621		96.3	-0.39
rs12927980	AA	221	3.8	1197	87.6	-0.75	0.70	1196	96.5	-	0.080		
	GG	1184	3.9		127	88.4			0.85	126		98.7	2.47
	GT	125	4.4		3	87.3			0.27	3		95.7	-1.26
rs11075290	CC	543	3.6	545	87.2	-	0.042	547	95.7	-	0.002		
	CT	553	4.0		560	87.4			0.27	556		97.0	1.23
	TT	211	4.7		216	89.2			1.92	216		98.6	2.95
rs246241	GG	746	4.0	756	87.8	-	0.52	754	96.9	-	0.60		
	GT	492	4.0		493	87.5			-0.17	493		96.3	-0.52
	TT	75	3.3		78	86.4			-1.09	78		97.1	0.38
rs212093	GG	460	3.6	468	88.2	-	0.36	467	96.7	-	0.78		
	GA	638	4.2		638	87.3			-0.94	638		96.9	0.14
	AA	213	3.8		219	87.5			-0.85	218		96.3	-0.45
<i>ABCC2 (MRP2)</i>													
rs717620	CC	1056	3.8	1066	87.8	-	0.52	1065	96.8	-	0.41		
	CT	244	4.4		249	87.2			-0.23	248		96.0	-0.72
	TT	13	7.6		12	85.1			-2.09	12		98.7	1.92
rs2756103	AA	679	3.8	689	87.8	-	0.63	685	96.9	-	0.60		
	AC	523	4.0		528	87.5			-0.34	529		96.3	-0.51
rs7393105	CC	111	4.4	110	86.9	-0.87	0.88	111	97.0	0.07	0.47		
	AA	545	3.9		608	87.5			-0.28	608		96.3	-0.73
	AC	605	3.9		165	87.4			-0.22	166		96.9	-0.11
rs2273697	GG	843	3.9	849	87.8	-	0.64	849	96.6	-	0.89		
	CC	163	4.2		428	87.3			-0.31	427		96.9	0.37
	GA	421	3.9		50	87.3			-0.52	49		96.2	-0.45
<i>ABCB1 (MDR1)</i>													
rs2032582	GG	840	3.6	848	87.5	-	0.077	848	96.9	-	0.11		
	GT	357	4.4		364	87.7			0.09	364		96.1	-0.86
	TT	56	5.6		54	90.8			1.73	54		99.1	1.75
rs2235035	GG	821	3.9	832	87.7	-	0.96	832	96.3	-	0.30		
	GA	430	4.0		434	87.5			-0.15	434		97.3	0.88
	AA	62	4.3		61	87.7			0.02	59		97.4	0.94
rs10274587	GG	929	4.0	942	87.5	-	0.54	939	96.8	-	0.74		
	GA	341	3.8		340	87.6			0.07	341		96.3	-0.56
	AA	42	3.6		44	89.3			1.85	44		97.2	0.45
rs1202169	TT	564	3.7	572	87.5	-	0.92	570	97.2	-	0.32		
	TC	599	4.0		606	87.7			-0.11	606		96.3	-0.95
	CC	148	4.7		147	87.9			-0.18	147		96.4	-0.87
rs1202171	AA	745	4.1	753	87.6	-	0.63	749	96.7	-	0.99		
	AT	499	3.7		498	87.4			-0.17	500		96.7	-0.05
	TT	69	4.0		76	88.7			1.03	76		96.6	0.09
rs10276499	TT	507	4.4	510	87.9	-	0.065	509	96.3	-	0.27		
	TC	602	3.8		610	87.9			0.23	609		97.2	0.86
	CC	203	3.2		206	86.1			-1.37	206		96.1	-0.13

218 ^a The hair Hg model is unadjusted, so the p-values test for differences between the three
 219 means. The homozygote for the most common allele is used as reference. The beta
 220 coefficients (β) for the MDI and PDI models are adjusted for child sex, maternal age at
 221 delivery, presence of two parents in the household, Hollingshead socioeconomic score, and
 222 child age at testing. The p-values from the sequential sum of squares test for differences in the
 223 effect of SNP and are unadjusted for the other covariates. SNP: single nucleotide
 224 polymorphism.
 225

226 In our analysis of maternal ABC genotypes in relation to the neurodevelopment of their
227 children we found that only *ABCC1* rs11075290 was significantly associated with MDI and
228 PDI (Table 3). Mothers with the CC variant had the lowest hair Hg concentrations, but the
229 children born to them showed poorer performance. Their children averaged 2 points lower for
230 the MDI and 3 points lower for the PDI than children born to mothers with TT variant who
231 had higher hair Hg. No other clear patterns of associations were found between the BSID-II
232 outcomes and ABC SNPs.

233
234

235 **4. Discussion**

236 This study suggests that genetic differences in ABC transporters influence human MeHg
237 body burden; seven out of 15 ABC SNPs genotyped were significantly associated with
238 concentrations of Hg in hair among mothers in a cohort with high fish consumption and a
239 mean hair Hg concentration of 3.9 ppm. Among the various maternal ABC transporter
240 genotypes that were associated with Hg in hair, only one SNP, in *ABCC1* (rs11075290), was
241 associated with child BSID scores. No other clear pattern of association with developmental
242 outcomes was observed. Based on the strong association of ABC genotype with maternal hair
243 Hg concentrations we predict that the ABC transporter genotype of the child may also play a
244 role in the child's MeHg body burden. Importantly, these transporters are intrinsic
245 components of the placenta and the blood-brain barrier and thus could influence brain
246 exposure to MeHg in the developing child.

247

248 It should however be stressed that ABC transporters transports a number of different
249 molecules and are thus not specific for MeHg transport. Consequently, the associations
250 between ABC transporters and neurodevelopment may be blurred by the transport of other
251 compounds that may influence neurodevelopmental outcomes. The involvement of
252 *ABCC1*/MRP1 in human MeHg distribution and elimination was suggested by findings from
253 experimental studies. Knocking down *ABCC1* in fruit flies resulted in accumulation of MeHg
254 and greater developmental toxicity measured as eclosion (Prince et al. 2014) and over-
255 expression of the *ABCC1*/MRP1 protein in mice resulted in decreased accumulation of MeHg
256 in brain and liver (Toyama et al. 2011). However, we do not presently know the role of
257 *ABCC1*/MRP1 in altering MeHg body burden in humans, and particularly during pregnancy.
258 The *ABCC1*/MRP1 protein is expressed in several tissues that are critical in absorption,
259 distribution and excretion of MeHg, both in the mother and the fetus. *ABCC1*/MRP1

260 expression occurs in many tissues (www.proteinatlas.org), including the placenta (Aye et al.
261 2007), the blood-brain barrier (Leslie et al. 2005), the intestine (Berggren et al 2007) and the
262 hair follicle (Haslam et al. 2013). Its expression in the hair follicle is of particular relevance
263 when considering hair as the biomarker of exposure as used in this study. During hair
264 synthesis MeHg from the bloodstream is transported to keratinocytes in the follicle that,
265 subsequent to differentiation and death, are incorporated in the matrix of the growing hair
266 shaft (Kempson and Lombi 2011; Zareba et al. 2008). Nonetheless, information about the
267 apical/basal orientation of ABCC1/MRP1 within the hair follicle, and other tissues, is limited
268 and it is therefore difficult to predict how the genotype ultimately influences the kinetics of
269 MeHg transport and distribution in various compartments of the body.

270

271

272 Among the 15 SNPs investigated, just one SNP, rs11075290 in *ABCC1*, was associated with
273 both maternal hair Hg concentrations and child BSID scores. It is positioned in the first
274 intron of the *ABCC1* gene in a promoter flanking region. The C allele creates a CpG site,
275 which can be methylated and potentially result in lower expression of *ABCC1*. Nevertheless,
276 the function of this SNP has not been tested *in vitro* and is uncertain. Its impact on MeHg
277 transport and distribution at the level of the placental/fetal circulation remains unclear. Thus,
278 in contrast to the strong association between maternal ABC genotype and a maternal mercury
279 disposition phenotype, the impact at the level of developmental phenotype in the offspring is
280 less certain. The association with developmental phenotype is likely to be determined also by
281 the child's ABC genotype. Future analyses incorporating the children's ABC SNPs are
282 needed to determine if there are susceptible sub-populations that can be identified. It is also
283 possible that our findings reflect linkage disequilibrium of this SNP with other functional
284 SNPs not yet characterized.

285

286 We did not include Hg in hair in the model for the association between ABC genotype and
287 neurodevelopment. If ABC genotype would have been clearly associated with
288 neurodevelopmental outcomes then it would have been appropriate to conduct a mediation
289 model including Hg in hair. However, in the absence of clear associations of ABC genotype
290 with neurodevelopmental outcomes as well as Hg in hair and neurodevelopmental outcomes
291 (Davidson et al. 1998; Myers et al. 2003; Strain et al. 2015), and recognizing the broad
292 functions of ABC transporters, we did not conduct a mediation analysis that included Hg in
293 hair as a covariate in the model.

294

295 We found that additional SNPs in *ABCC1*, *ABCC2*, and *ABCB1* were also associated with hair
296 Hg, and are thus candidates to explore for a role in MeHg transport, distribution and
297 elimination. The ABC SNPs varied in allele frequency when comparing NC2 with individuals
298 from Hapmap with European and African ancestry: alleles associated with elevated Hg in hair
299 were in general higher in frequency in Hapmap individuals with European ancestry. Further
300 studies on the role of ABC SNPs for MeHg concentrations in different tissues, such as blood
301 and brain, will facilitate understanding of individual differences in Hg distribution and
302 elimination. Such studies will potentially help us to identify susceptible groups. An earlier
303 study found that the promoter-associated rs717620 (<http://www.ensembl.org>) was correlated
304 with urinary Hg concentrations (a marker of inorganic Hg) among gold miners (Engstrom et
305 al. 2013). The rs717620 A-allele carriers had higher Hg concentrations in urine than
306 individuals with the GG genotype. A parallel pattern was seen for hair Hg in this study, where
307 the A-allele was associated with higher hair Hg concentrations, suggesting that this SNP may
308 act to influence overall transport and elimination of mercury.

309

310 Strengths of this study include the large well-characterized cohort and extensive information
311 about sequence variation in some genes encoding potential transporters for MeHg. The lack of
312 genetic data for the children is a limitation, but further studies aim to characterize more fully
313 the contribution of child genotype on MeHg body burden and cognitive outcomes.

314

315 **5. Conclusions**

316 We found that SNPs in genes coding for three ABC transporter genes are associated with
317 maternal hair Hg concentrations in a population consuming large quantities of ocean fish.
318 These findings suggest that genetics may play a role in determining maternal, and thus
319 prenatal, MeHg exposure. While consistent associations with BSID outcomes were not
320 observed, we cannot exclude the possibility that ABC transporter genes play a role in
321 influencing child MeHg body burden and possibly neurodevelopmental outcomes. Further
322 studies are needed to explore the functional consequences of these polymorphisms,
323 importantly, extending the study to the child's ABC transporters, to fully understand effects
324 on MeHg toxicokinetics and MeHg-related effects on neurodevelopment.

325

326 **Acknowledgments**

327 We gratefully acknowledge the participation of all women and children who took part in the
328 study and the nursing staff from the Child Development Centre, Seychelles for their
329 assistance with data collection.

330 Supported by the US National Institute of Health (grants R01-ES010219 and P30-ES01247),
331 The Swedish Research Council for Health, Working Life and Welfare (FORTE), Swedish
332 Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS),
333 and in-kind support from the Government of Seychelles. The study sponsors had no role in
334 the design, collection, analysis, or interpretation of data, in the writing of this article, or in the
335 decision to submit the article for publication.

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