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Original Contribution

A Birth Cohort Study on the Genetic Modification of the Association of Prenatal Methylmercury With Child Cognitive Development

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Genetic predisposition might affect neurodevelopmental outcomes of prenatal methylmercury exposure. We examined suspected heterogeneities for modification of exposure-related neurodevelopment in children from the Avon Longitudinal Study of Parents and Children (1991–2000), Bristol, United Kingdom. A subgroup ($n = 1,127$ from a pilot study and 1,045 from the present study) was identified based on the availability of the mercury concentration of cord tissue as a measure of prenatal methylmercury exposure, data on 247 single-nucleotide polymorphisms (SNPs), and Wechsler Intelligence Scale for Children intelligence quotient (IQ) scores. Log₁₀-transformed mercury concentration was positively associated with IQ, but adjustment for confounding cofactors attenuated this association. A finding of enhanced interaction with methylmercury was replicated in this study for the minor allele of rs1042838 (progesterone receptor) ($\beta = -11.8$, 95% confidence interval: $-23.0, -0.6$; P for interaction = 0.004) and weakly for rs662 (paraoxonase 1) ($\beta = -3.6$, 95% confidence interval: $-11.4, 4.3$; $P = 0.117$). In the joint sample, new interacting single-nucleotide polymorphisms were discovered in relation to superoxide dismutase 2, ATP binding cassette subfamily A member 1, and metallothionein 1M genes. While the low-level prenatal exposure to methylmercury was not associated with child cognition, progesterone receptor rs1042838 minor alleles revealed a negative association of mercury exposure with IQ.

ALSPAC; cognitive functions; genes; mercury; neuropsychological development; population-based birth cohort; pregnancy; SNPs

Abbreviations: *ABCA1*, ATP binding cassette subfamily A member 1; ALSPAC, Avon Longitudinal Study of Parents and Children; *BDNF*, brain-derived neurotrophic factor; IQ, intelligence quotient; *MT1M*, metallothionein 1M; *PGR*, progesterone receptor; *PON1*, paraoxonase 1; SNP, single-nucleotide polymorphism; *SOD2*, superoxide dismutase 2; *TF*, transferrin; WISC-III, Wechsler Intelligence Scale for Children, Third Edition.

Methylmercury exposure can impair neurodevelopment, mainly during prenatal stages, potentially leading to permanent cognitive deficits (1–3). Prevention of developmental exposure to methylmercury aims at protecting against doses that are associated with declines in average cognitive performance (4), but recent studies suggest that common genetic heterogeneities could affect neurotoxic responses (5–7). In a pilot study of 1,127 members of the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, Bristol, United Kingdom, we identified 4 single-nucleotide polymorphisms (SNPs) in genes for brain-derived neurotrophic factor (*BDNF*), paraoxonase 1 (*PON1*), transferrin (*TF*), and progesterone receptor

(*PGR*), where presence of the minor allele was associated with greater methylmercury-linked cognitive deficits at the low background exposure of this population (8). The study considered 247 candidate SNPs involved in the potential biological pathways of prenatal methylmercury neurotoxicity, including those implicated in brain development, neurotransmitter metabolism, cholesterol metabolism, iron regulation, and peroxidative defense (8).

In order to determine the overall impact of genetic predisposition on methylmercury neurotoxicity, we have now obtained data from 1,045 additional cohort members. We first sought replication of the potential modifying effect of the 4 SNPs

previously identified and then explored the possible impact of other candidate SNPs in the overall sample ($n = 2,172$).

Seafood intake is the main source of methylmercury exposure (9, 10), but particularly fatty fish is rich in essential nutrients, such as long chain fatty acids (PUFAs), selenium, and vitamin D, that are necessary for neurodevelopment (11). These potentially beneficial factors are related to the frequency of seafood intake and thus tend to correlate with methylmercury exposure, thereby potentially exerting a confounding role on the methylmercury–cognitive function association (12). Thus, when statistically controlling for these confounding factors (13), adverse methylmercury–outcome associations might become stronger (9, 14).

In the present study, we analyzed the impact of relevant SNPs on the association between prenatal methylmercury exposure and child cognitive deficits at age 8 years while considering the potential confounding role of maternal socioeconomic position and seafood diets during pregnancy.

METHODS

Participants

ALSPAC is an ongoing longitudinal cohort study designed to investigate the determinants of development, health, and disease during childhood and beyond (15–17). Pregnant women, residing in the former Avon health authority area in Southwest England, with an expected delivery date between April 1, 1991, and December 31, 1992, were eligible to participate in the study. A cohort of 14,541 pregnant women was established, resulting in 13,988 children who were alive at 12 months of age. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the local research ethics committees. The study website contains details of all the data that are available through a fully searchable data dictionary and variable search tool (18). Based on the availability of SNPs and an intelligence quotient (IQ) score, biobanked slices of umbilical cords were analyzed for the total mercury concentration as a measure of the prenatal methylmercury concentration (19). A total of 2,172 cohort members were included, of whom 1,127 belonged to the pilot study (8), while an additional 1,045 became available for the present study. All subjects had available data on SNPs within relevant genes (8), as well as IQ scores at age 8 years. Final models with the covariate variables included 1,723 complete cases, of which 933 originated from the pilot study and 790 were newly identified. See Figure 1 for a flow chart of participants in this study. Inverse-probability weighting, to control for potential selection bias induced by restricting the analysis to complete cases (i.e., individuals with no missing values) (20), was applied as sensitivity analyses that are shown in Web Tables 1–5 (available at <https://academic.oup.com/aje>).

Mercury measurement

Cord samples were taken by the midwife at birth and frozen at -20°C . Samples were defrosted briefly to divide them into several 1-cm slices, which were then stored at -20°C . After freeze-drying the cord tissue slices, the total mercury dry-weight

concentration was determined using a direct mercury analyzer (DMA-80; Milestone, Inc., Shelton, Connecticut) at the University of Southern Denmark (8). The dry-weight measure is known to have an appropriate precision (21).

SNP genotyping

We chose candidate genes belonging to pathways considered of possible relevance after systematically reviewing the literature. Gene candidates were selected based on their possible role in pathways associated with methylmercury toxicity (8). They relate to 4 major biological pathways considered important to neurodevelopment or metal neurotoxicity: 1) brain development and neurotransmitter metabolism, 2) cholesterol metabolism, 3) iron regulation, and 4) peroxidative defense. We selected polymorphisms with a minor allele frequency of at least 10%, and the SNPs chosen have been previously described (8).

ALSPAC children were genotyped using the Illumina HumanHap550 (Illumina, Inc., San Diego, California) quadchip genotyping platform. Standard quality-control methods were performed and have been previously described in the scientific literature (22). Genotypic data were subsequently imputed using MACH (23) and phased haplotype data from Hap-Map CEU (r22) (24). Data from genotyped and imputed SNPs (using the most likely genotype) were extracted for 247 SNPs.

Neuropsychological data

When the children were 8 years of age, a short form of the Wechsler Intelligence Scale for Children, Third Edition (WISC-III) (25), was used to assess IQ. Using lookup tables provided in the WISC-III manual, age-scaled scores were obtained from the raw scores for each subtest, and total, performance, and verbal IQ scores were calculated. The mean age at assessment was 8.5 years (standard deviation, 0.3 years). The testers were trained psychologists who were overseen by a senior psychologist with long experience of psychometric testing within the study. She observed each tester, met with the group regularly to discuss the precise administration of each subtest, and checked their scoring (26, 27).

Selected covariate variables

We used the same covariates as in our pilot study (8). Briefly, the following variables were taken into account in the associative models between the exposure and outcome: sex, age at WISC-III assessment, and WISC-III examiner. Several covariates were retained in the final model because of prior knowledge that they were related to the exposure. Omega-3 fatty acid intake estimates were derived from a food-frequency questionnaire on seafood consumption during pregnancy (26, 28). Maternal age, maternal smoking during pregnancy, parity, house ownership status, parental education, and social class were recorded during pregnancy. The final models previously published (8) also adjusted for the healthy component of the diet during pregnancy and estimated the children's processed component of the diet at age 8 years. For this study, these 2 covariate variables were

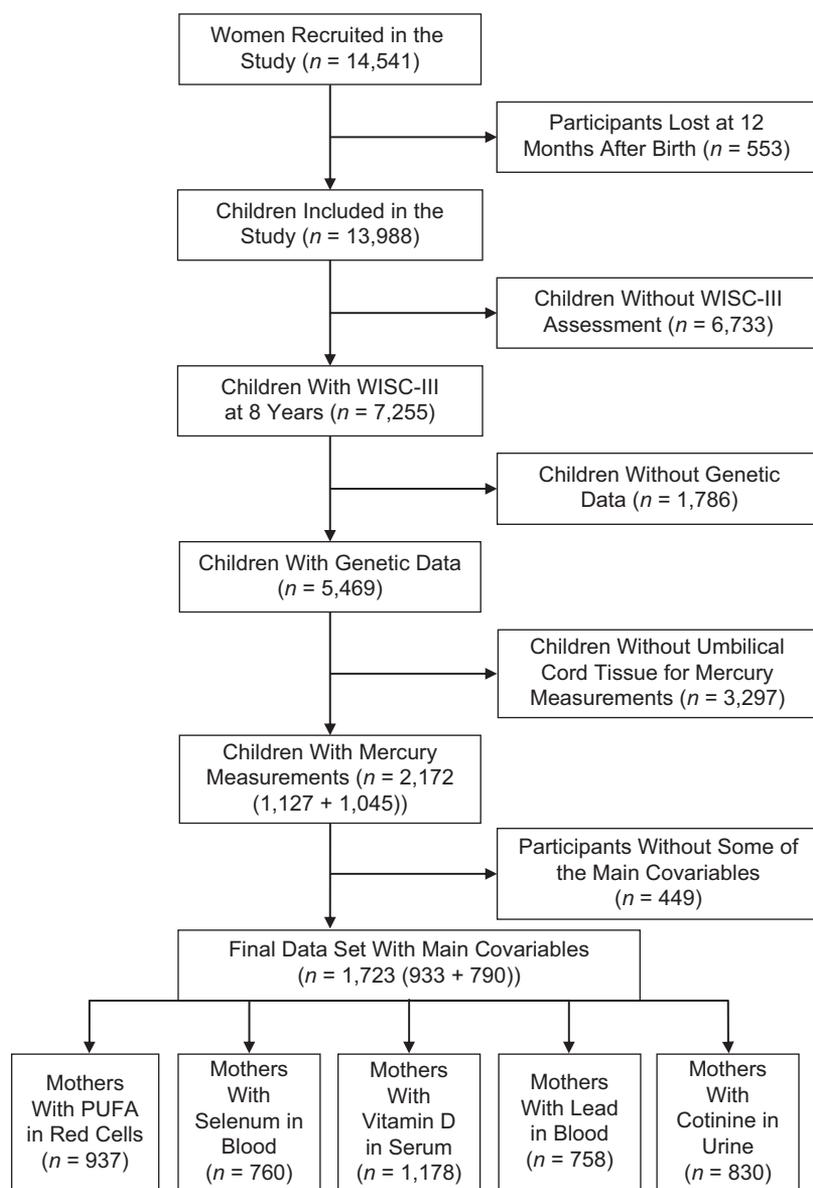


Figure 1. Main phases of the study and flowchart of the population, Avon Longitudinal Study of Parents and Children, United Kingdom, 1991–2000. PUFA, polyunsaturated fatty acid; WISC-III, Wechsler Intelligence Scale for Children, Third Edition.

excluded due to incomplete observations (lacking data: pilot study $n = 90$; extended study $n = 137$) and marginal impact on the final models (shown as secondary analyses). Allowing inclusion of these subjects caused only slight changes in the findings, compared with pilot sample analyses previously published (8). The extended set of samples showed higher prevalence of lower maternal social class than the pilot subsample (data not shown), and adjustment for maternal social class was retained in all models. Furthermore, in sensitivity analyses, we included biomarkers related to seafood intake and other pollutants: maternal whole blood selenium concentration during pregnancy (29), maternal blood lead concentration during pregnancy (30), maternal serum 25(OH)D concentration (reflecting both vitamin D₂ and vitamin D₃)

during pregnancy (31), and maternal urine cotinine concentration during pregnancy (32). Maternal long-chain fatty acid concentrations in red-cell membranes from blood samples during pregnancy (33), docosahexaenoic acid and arachidonic acid, were measured in a subgroup and included in sensitivity analyses. Figure 1 shows numbers of participants with these variables available.

Statistical analysis

Except when attempting to replicate the findings for the 4 SNPs identified in the pilot study (8), all quantitative analyses were based on complete data sets comprising both subsamples. Mercury concentrations were normally distributed after log₁₀

transformation, and crude and adjusted linear regressions were therefore used to assess the relationship between methylmercury exposure and child WISC-III outcomes.

Eleven SNPs were removed due to (unexpected) low minor allele frequency (<10%) or poor imputation quality ($R^2 < 0.8$) so that the genetic analyses ultimately included 236 SNPs. The 4 SNPs identified in the previous study, *TF* rs3811647, *PONI* rs662, *BDNF* rs2049046, and *PGR* rs1042838, were first examined for possible replication in the extended sample. As a heuristic, in the total sample, the SNPs were scanned for “main effects” using a nominal selection level for further investigation of a P value ≤ 0.05 for association with child IQ outcomes and methylmercury exposure. The “main effects” were assessed using crude linear regression models assuming an additive mode of inheritance (e.g., genotypes coded as 0, 1, 2). A total of 32 of the 236 SNPs passing this threshold were then further analyzed in an interaction model adjusting for the covariate variables. When testing interactions between SNPs and methylmercury, multiple comparisons were addressed by correcting nominal P values using Bonferroni criteria ($0.05/(32 \text{ SNPs}) = 0.0016$). ALSPAC genetic ancestry data was introduced in the final models to adjust for any ancestry confounding (34). Finally, inverse-probability weighting was applied in sensitivity analyses (20). All analyses were performed using Stata, release 12 (StataCorp LLC, College Station, Texas).

RESULTS

The overall mercury mean concentration in umbilical cord was 25.2 (standard deviation, 12.7) ng/g dry weight. This

corresponds to a cord-blood mercury concentration of 2.70 $\mu\text{g/L}$ (19). The mercury concentration showed associations with the study covariate variables similar to the previous study, and adding 1,045 cases did not change the previous descriptive results. Higher strata of maternal social class and higher levels of omega-3 fatty acid intake during pregnancy remained reliably associated with cord-mercury concentrations (Web Table 1). The Spearman correlation coefficient between the mercury concentration and the calculated omega-3 fatty acid intake from seafood consumption during pregnancy was $\rho = 0.45$; it was $\rho = 0.26$ between the mercury concentration and the ratio between docosahexaenoic acid and arachidonic acid concentrations in maternal pregnancy red-cell membranes.

Crude mercury associations with WISC-III outcomes showed positive coefficients that were very substantially attenuated by adjustment for sociodemographic and omega-3 fatty acid intake variables. This association pattern was similar in the extended and joint sample analyses (Table 1). No changes were observed if we included maternal red-cell membrane docosahexaenoic acid–arachidonic acid concentration ratio in the models (data not shown). The final models for the joint sample stratified by maternal social class showed an interaction in which higher social-class strata showed the expected negative coefficient for the mercury association with the child’s performance IQ (Web Table 2).

Sensitivity analyses of the mercury and WISC-III outcome associations are shown in Table 2. The full models in Table 1 additionally and separately adjusted for several biomarkers: pregnancy blood selenium, blood lead, serum vitamin D, and urine cotinine concentrations. No material changes were

Table 1. Adjusted Regression Coefficients for Cord-Mercury Concentration as a Predictor of 8-Year Psychometric Outcomes in the New and Combined Subgroups, Avon Longitudinal Study of Parents and Children, United Kingdom, 1991–2000

WISC-III Score	Model 1 ^a			Model 2 ^b			Model 3 ^c		
	No.	β^d	95% CI ^d	No.	β^d	95% CI ^d	No.	β^d	95% CI ^d
Extended sample (new group)	1,051			795			792		
Total IQ		11.6	6.6, 16.4		3.5	-2.0, 9.0		2.6	-3.4, 8.6
Verbal IQ		12.8	7.9, 17.8		3.2	-2.3, 8.8		1.9	-4.1, 7.9
Performance IQ		7.2	2.1, 12.3		2.8	-3.3, 8.8		2.6	-4.0, 9.1
Joint sample (pilot group + new group)	2,281			1,813			1,808		
Total IQ		12.1	8.9, 15.2		3.0	-0.4, 6.4		2.6	-1.1, 6.4
Verbal IQ		13.3	10.1, 16.6		3.4	-0.1, 6.9		2.9	-0.9, 6.8
Performance IQ		7.5	4.2, 10.8		1.8	-2.0, 5.5		1.6	-2.5, 5.7
Joint sample complete cases	1,808			1,808			1,808		
Total IQ		12.1	8.6, 15.6		3.0	-0.4, 6.5		2.6	-1.1, 6.4
Verbal IQ		12.9	9.2, 16.5		3.3	-0.2, 6.8		2.9	-0.9, 6.8
Performance IQ		8.0	4.3, 11.8		1.8	-1.9, 5.6		1.6	-2.6, 5.7

Abbreviations: CI, confidence interval; IQ, intelligence quotient; WISC-III, Wechsler Intelligence Scale for Children, Third Edition.

^a Adjusted for sex, age, and examiner. These analyses included subjects without genetic data, resulting in a slightly larger sample size than the genetic data analyses.

^b Additionally adjusted for parental education level, maternal age, smoking during pregnancy, social class, parity, and house ownership status.

^c Additionally adjusted for maternal estimated omega-3 intake (omega-3 intake estimated from seafood intake).

^d β and 95% CI for \log_{10} (cord-mercury (ng/g)).

Table 2. Adjusted Regression Coefficients for Cord-Mercury Concentration as a Predictor of the 8-Year Psychometric Outcomes in the Combined Group in Sensitivity Analyses for Maternal Selenium, Lead, Vitamin D, and Cotinine Concentration During Pregnancy, Avon Longitudinal Study of Parents and Children, United Kingdom, 1991–2000

Measure	WISC-III Score						
	No.	Total IQ		Verbal IQ		Performance IQ	
		β^a	95% CI ^a	β^a	95% CI ^a	β^a	95% CI ^a
Selenium ^b							
Model 1 ^c	760	-0.7	-6.5, 5.1	-0.8	-6.8, 5.2	-0.7	-7.2, 5.8
Model 2 ^d	760	-0.3	-6.2, 5.6	0.1	-6.1, 6.1	-1.1	-7.7, 5.5
Lead ^b							
Model 1 ^c	758	-0.7	-6.6, 5.1	-0.8	-6.8, 5.2	-0.8	-7.3, 5.7
Model 3 ^e	758	-1.0	-6.8, 4.9	-1.1	-7.1, 4.9	-0.9	-7.4, 5.6
Vitamin D total ^b							
Model 1 ^c	1,178	0.9	-3.7, 5.5	1.4	-3.3, 6.1	0.3	-4.8, 5.4
Model 4 ^f	1,178	1.0	-3.7, 5.6	1.4	-3.3, 6.1	0.5	-4.6, 5.6
Cotinine ^b							
Model 1 ^c	830	-1.5	-7.0, 4.0	-1.9	-7.6, 3.8	-0.7	-7.0, 5.5
Model 5 ^g	830	-1.5	-7.0, 4.0	-1.9	-7.6, 3.8	-0.7	-7.0, 5.6

Abbreviations: CI, confidence interval; IQ, intelligence quotient; WISC-III, Wechsler Intelligence Scale for Children, Third Edition.

^a β and 95% CI for \log_{10} (cord-mercury (ng/g)).

^b Selenium (median, 111 ug/l); lead (median, 3 ug/L); vitamin D total (median, 64 nmol/L); cotinine (median, 27 ng/mL).

^c Basic model adjusted for sex, age, examiner, parental education level, maternal age, smoking during pregnancy (except for cotinine models), social class, parity, house ownership status, and estimated omega-3 intake (omega-3 intake estimated from seafood intake).

^d Additionally adjusted for maternal selenium blood concentration during pregnancy.

^e Additionally adjusted for maternal lead blood concentration during pregnancy.

^f Additionally adjusted for maternal total vitamin D blood concentration during pregnancy.

^g Additionally adjusted for maternal cotinine urine concentration during pregnancy.

observed in the results after inclusion of these covariate variables. Due to a high degree of missingness of these new covariables, we applied corrections by inverse-probability weighting, and the results were unchanged (Web Table 3).

Mercury concentrations for selected child genotypes are shown in Table 3. The first 4 SNPs were previously selected in the pilot study on gene-mercury interactions and child IQ, while the additional SNPs emerged after examination of the combined sample. No SNP showed a clear association with mercury concentrations. Likewise, no SNP was associated with omega-3 fatty acid intake from seafood consumption during pregnancy (data not shown).

The results in Table 4 show gene-environment interaction analyses—within the pilot, extended, and joint sample materials—of mercury associations with the WISC-III outcomes for the 4 genotypes previously identified (8). *PGR* rs1042838 presented a similar interaction pattern to that previously reported. *PONI* rs662 showed a similar but weaker interaction pattern. *TF* rs3811647 and *BDNF* rs2049046 associations were not replicated in this extended sample. The *P*-for-interaction value for *PGR* rs1042838 even passed a Bonferroni correction in the joint sample. Furthermore, as shown in Table 5, apparently novel findings were identified in the joint sample: superoxide dismutase 2 (*SOD2*) rs5746136, ATP binding cassette subfamily A member 1 (*ABCA1*) rs4149268, *ABCA1* rs3890182, and metallothionein 1M (*MT1M*) rs2270836 tended to show major allelic variants with lower or negative mercury

coefficients in the stratification analyses, in contrast to the minor alleles showing negative coefficients in the heterogeneities first identified.

All the findings presented in Tables 4 and 5 were unchanged after adjusting for ALSPAC genetic ancestry (data not shown). The same models were repeated for the joint samples with inverse-probability weighting corrections, and again the results were unchanged (see Web Table 4). We further observed a linear and monotonic dose-response pattern with the mercury–total IQ association from *PGR* variants in generalized additive models (see Web Figure 1). Furthermore, no changes were observed with the inclusion of additional covariates, such as maternal healthy diet score during pregnancy and child processed diet score (Web Table 5).

DISCUSSION

In this extended subgroup within the ALSPAC prospective cohort study, the mercury concentration in cord tissue was not associated with 8-year cognitive development. Substantial attenuation of the positive coefficient was observed after adjustment for maternal social class and estimated omega-3 fatty acid intake from seafood during pregnancy, and the small, residual positive association could well be null or inverse if plausible levels of measurement error in the confounders and exposure were assumed (35). No substantial

Table 3. Cord-Mercury Concentrations According to Selected Child Genotypes in the Pilot and the Combined Groups, Avon Longitudinal Study of Parents and Children, United Kingdom, 1991–2000

Gene	SNP	Major/Minor Allele	Cord-Mercury Concentration (ng/g)						P Value ^a
			Homozygous for the Major Allele		Heterozygous		Homozygous for the Minor Allele		
			No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	
<i>Pilot SNPs (Discovery From Pilot Sample Analyses)</i>									
<i>TF</i>	rs3811647	G/A	1,004	26 (18)	1,010	25 (13)	256	24 (11)	0.17
<i>PON1</i>	rs662	T/C	1,180	25 (12)	929	25 (19)	161	24 (13)	0.73
<i>BDNF</i>	rs2049046	T/A	679	26 (18)	1,121	25 (12)	470	25 (18)	0.47
<i>PGR</i>	rs1042838	G/T	1,598	25 (16)	612	25 (12)	60	27 (12)	0.70
<i>New SNPs (Discovery From Joint Sample Analyses)</i>									
<i>SOD2</i>	rs5746136	G/A	1,081	25 (18)	993	25 (13)	196	25 (12)	0.82
<i>ABCA1</i>	rs4149268	C/T	931	25 (12)	1,016	26 (18)	323	24 (12)	0.41
<i>ABCA1</i>	rs3890182	G/A	1,659	26 (16)	568	25 (13)	43	24 (11)	0.33
<i>MT1M</i>	rs2270836	C/T	842	26 (19)	1,029	25 (13)	370	25 (11)	0.50

Abbreviations: *ABCA1*, ATP binding cassette subfamily A member 1; *BDNF*, brain-derived neurotrophic factor; Hg, mercury; *MT1M*, metallothionein 1M; *PGR*, progesterone receptor; *PON1*, paraoxonase 1; SD, standard deviation; SNP, single nucleotide polymorphism; *SOD2*, superoxide dismutase 2; *TF*, transferrin.

^a One-way analysis of variance (ANOVA) P value between cord-slice Hg concentration and the SNP variants.

change in the association was observed after adjustment for maternal pregnancy selenium and vitamin D concentrations. Because fish intake is the main human source of methylmercury exposure (36), the low exposure levels might be a result of low-frequency fish intake in the ALSPAC cohort, in accord with the food habits of England's general population

(27). Studies on prenatal exposure to methylmercury have observed stronger adverse associations with mercury after statistically controlling for the opposing associations with fish intake and omega-3 fatty acids (8, 9, 13, 37). Other nutrients linked to fish intake, such as selenium and vitamin D, might confer healthy neurodevelopment (11, 38–40), but

Table 4. Adjusted Regression Coefficients^a for the Cord-Mercury Concentration (ng/g) as a Predictor of Intelligence Quotient According to Selected Genotypes (Pilot, Extended, and Combined Groups), Avon Longitudinal Study of Parents and Children, United Kingdom, 1991–2000

Gene	Log ₁₀ (Hg), Pilot Sample ^b				Log ₁₀ (Hg), Extended Sample				Log ₁₀ (Hg), Joint Sample			
	No.	β	95% CI	P for Interaction	No.	β	95% CI	P for Interaction	No.	β	95% CI	P for Interaction
rs3811647 (<i>TF</i>) 11	402	4.8	−2.8, 12.3	0.142	358	6.7	−2.4, 15.8	0.288	760	6.2	0.4, 11.9	0.672
rs3811647 (<i>TF</i>) 12	427	2.8	−5.3, 10.9		341	−4.7	−14.1, 4.7		768	0.9	−5.1, 7.0	
rs3811647 (<i>TF</i>) 22	104	−7.1	−24.5, 10.3		91	5.9	−12.4, 24.2		195	0.9	−11.3, 13.1	
rs662 (<i>PON1</i>) 11	494	7.5	0.5, 14.5	0.156	404	8.6	−1.0, 18.2	0.117	898	7.8	2.2, 13.3	0.054
rs662 (<i>PON1</i>) 12+22 ^c	439	−2.3	−10.0, 5.5		386	−3.6	−11.4, 4.3		825	−2.2	−7.7, 3.3	
rs1042838 (<i>PGR</i>) 11	648	6.7	0.4, 12.9	0.049	549	7.0	−0.3, 14.3	0.004	1,197	7.0	2.3, 11.7	0.001
rs1042838 (<i>PGR</i>) 12+22 ^c	285	−5.3	−14.9, 4.3		241	−11.8	−23.0, −0.6		526	−7.0	−14.1, 0.0	
rs2049046 (<i>BDNF</i>) 11	274	8.2	−1.9, 18.3	0.446	240	0.8	−9.5, 11.1	0.653	514	4.8	−2.3, 11.9	0.986
rs2049046 (<i>BDNF</i>) 12	473	3.6	−3.8, 10.9		381	−0.1	−9.7, 9.6		854	3.1	−2.7, 8.9	
rs2049046 (<i>BDNF</i>) 22	186	−6.2	−17.7, 5.2		169	7.8	−4.5, 20.1		355	1.4	−6.7, 9.5	

Abbreviations: *BDNF*, brain-derived neurotrophic factor; CI, confidence interval; Hg, mercury; *PGR*, progesterone receptor; *PON1*, paraoxonase 1; *TF*, transferrin.

^a All multivariate linear regression models adjusted for sex, age, examiner, parental education level, maternal age, smoking during pregnancy, social class, parity, house ownership status, and estimated omega-3 intake (omega-3 intake estimated from seafood intake).

^b Results from the pilot sample were slightly different from previous published results (8), due to adding more observations here but not due to adding a different confounding structure (data not shown).

^c The alleles 12 and 22 were combined into a unique category due to low number of observations (22 alleles <10% of the total sample).

Table 5. Adjusted Regression Coefficients^a for the Cord-Mercury Concentration (ng/g) as a Predictor of Total Intelligence Quotient According to Selected Genotypes, From a Joint Sample, Avon Longitudinal Study of Parents and Children, United Kingdom, 1991–2000

Genotype	Log ₁₀ (Cord-Slice Hg (ng/g))			
	No.	β	95% CI	P for Interaction
Cases with WISC-III IQ score available	1,723			
New SNPs (discovery from joint sample analyses)				
rs5746136 (<i>SOD2</i>) 11	797	0.9	−4.5, 6.3	0.082
rs5746136 (<i>SOD2</i>) 12+22 ^b	926	5.5	−0.2, 11.2	
rs4149268 (<i>ABCA1</i>) 11	695	−4.4	−10.7, 1.8	0.049
rs4149268 (<i>ABCA1</i>) 12	764	6.2	0.4, 12.0	
rs4149268 (<i>ABCA1</i>) 22	264	9.2	−1.6, 19.9	
rs3890182 (<i>ABCA1</i>) 11	1,257	1.2	−3.4, 5.8	0.038
rs3890182 (<i>ABCA1</i>) 12+22 ^b	466	8.4	0.8, 16.1	
rs2270836 (<i>MT1M</i>) 11	645	−1.2	−7.4, 5.1	0.054
rs2270836 (<i>MT1M</i>) 12	777	5.1	−0.8, 11.0	
rs2270836 (<i>MT1M</i>) 22	281	10.3	−0.1, 20.8	

Abbreviations: *ABCA1*, ATP binding cassette subfamily A member 1; CI, confidence interval; Hg, mercury; IQ, intelligence quotient; *MT1M*, metallothionein 1M; *SOD2*, superoxide dismutase 2; WISC-III, Wechsler Intelligence Scale for Children, Third Edition.

^a All multivariable linear regression models adjusted for sex, age, examiner, parental education level, maternal age, smoking during pregnancy, social class, parity, house ownership status, and estimated omega-3 intake (omega-3 intake estimated from seafood intake).

^b The alleles 12 and 22 were combined into a unique category due to low number of observations (22 alleles <10% of the total sample).

they did not appear to affect mercury-related cognitive deficits, as previously reported for selenium (13, 41, 42). Likewise, no change in the methylmercury coefficient was observed after adding biomarkers of other neurotoxicant exposures, such as lead in blood and urine-cotinine during pregnancy. The low seafood intake and the smaller sample size for these additional biomarker measurements might have prevented us from detecting any potential inverse confounding. Indeed, the biomarker analyses are not fully comparable, because samples with available biomarkers overlapped only little.

In relation to the genetic predisposition analyses, *PGR* rs1042838 SNPs findings were clearly replicated in the extended data set, and *PONI* rs662 received modest additional supportive evidence. New SNP results suggested additional interactions with methylmercury exposure and cognitive development in the joint sample (i.e., children with wild-type genetic variants of *SOD2* rs5746136, *ABCA1* rs4149268 and rs3890182, and *MT1M* rs2270836 showed greater vulnerability to methylmercury neurotoxicity). Thus, children with these variants showed evidence of methylmercury neurotoxicity that was not detectable in the cohort sample as a whole (i.e., without taking genetic predisposition into account).

The PROGINS-haplotype variant *PGR* rs1042838 is plausible as an indicator of greater vulnerability to methylmercury exposure. A biological explanation might be that progesterone appears to act as a neuroprotector. Thus, PROGINS-variant carriers tend to have more neurological problems along with lower progesterone levels (43). Specifically, rs1042838 minor

variant carriers might impair *PGR* protein functions by reducing the transcription or signaling of the *PGR* gene, thereby decreasing the effect of progesterone. Moreover, progesterone acts to oppose the effect of estrogen on glutamate homeostasis disruption (43, 44). Indeed, methylmercury is considered a metalloestrogen (45); in this sense, the rs1042838 minor variant carriers might be more vulnerable to mercury toxicity by increasing estrogen levels, on the one hand, and showing a reduced protective effect of progesterone on the glutamate homeostasis on the other. Glutamate is an important neurotransmitter involved in high-order cognitive functions, such as learning and memory, in the brain (46). However, there is a need to further understand the effects of the *PGR* rs1042838 modification with methylmercury and other environmental exposures. Of note, some recent studies detected an increased risk of breast cancer and endometrial cancer among rs1042838 minor variant carriers (44, 47), but they did not assess any potential effect modification by environmental factors.

The *PONI* rs662 association was weakly replicated with a dominant model in the joint sample analyses. Other *PONI* SNPs conferring predisposition to methylmercury neurotoxicity have also been reported (48). *PONI* codes for an enzyme that inhibits oxidation of lipoproteins. Such oxidative damage might be induced by methylmercury (8, 48). A recent study found prenatal methylation changes to be associated with prenatal mercury and cognitive performance with *PONI* (49). Because *BDNF* SNP (rs2049046) and *TF* SNP (rs3811647) associations could not be replicated in the joint

sample, we conclude that these variants are not important modulators of methylmercury toxicity in the present cohort, although we cannot rule out a potential role at higher exposure levels.

New SNPs were identified in the joint sample analyses of *SOD2*, *ABCA1* and *MTIM*, all previously considered in studies on genetic influence on mercury toxicokinetics (50). In an experimental study, mice were fed methylmercury-contaminated food, and the authors reported an exposure-stimulated expression in the brain of wild-type variants for mitochondrially encoded cytochrome b, cytochrome c oxidase subunit I, superoxide dismutase 1, *SOD2*, ATP-binding cassette subfamily B member 1A, and BCL2-associated X protein (51). Interestingly, we found more methylmercury vulnerability in the wild-type *SOD2* rs5746136 genotype. The *SOD2* protein binds to the superoxide byproducts of oxidative phosphorylation; it encodes a mitochondrial protein and binds 1 manganese ion per subunit. *SOD2* might also bind methylmercury molecules and thereby increase oxidative stress inside neuronal cells by disrupting mitochondrial functions (51). The *ABCA1* gene belongs to the superfamily of ABC transporters responsible for the active transport of various compounds across biological membranes (50). A recent population study found correlations between the hair-mercury concentration and alleles of this gene (52), and another study relying on cord-blood mercury measurements in 2 Mediterranean cohorts found similar results (7). Furthermore, *ABCA1* rs4149268 and rs3890182 were found to be related to lipid and obesity profiles (53–55). The increased expression of *ABCA1* SNPs wild-type variants might augment the passage of methylmercury molecules into neuronal cells and thereby further enhance the neurotoxic effect (50). Genes encoding metallothionein proteins have been explored because this protein actively binds metals via thiol groups in cysteine residues and protects against metal toxicity and oxidative stress in kidney, liver, and brain (50). A recent publication on 515 dental professionals found that *MTIM* polymorphisms were associated with mercury concentrations (56). Another study reported that male children's verbal learning scores showed a greater reduction at elevated urinary mercury concentrations if they were carriers of *MTIM* rs2270836 wild-type variant (5); our findings for the same SNP are in the same direction. The tentative novel findings we report here require replication if reliable evidence is to be generated to support their role in methylmercury toxicity.

The exposure measurement was based on freeze-dried cord tissue, which has shown good correlation to mercury concentrations in cord blood, considered to be the best indicator of prenatal methylmercury neurotoxicity (8, 19). The estimation of omega-3 fatty acid intake from seafood was based on food frequency questionnaires only. Although valid, such methods are probably less precise than the biomarker used for methylmercury exposure in this study. When a variable is measured with imprecision, some of the variance might be erroneously attributed to other associated variables that are assessed more precisely (8, 13, 57). In the regression analyses, positive associations of fish intake might therefore be attributed to the more precisely assessed methylmercury variable rather than the confounding positive factors themselves, with apparently absent methylmercury toxicity being artefactually observed (8, 14).

Furthermore, the results showed a tendency of mercury adverse associations with child IQ scores in higher social class strata (i.e., the group with higher exposure levels). In less-advantaged groups, risks of adverse exposures might vary much more, thereby contributing unexplained variance (8, 58).

In our study, we replicated findings of a genetic predisposition to methylmercury neurotoxicity, using the same interaction models previously applied in a separate subgroup from the same cohort. We validated the predisposition from *PGR* rs1042838 and, with less certainty, *PON1* rs662. None of the selected SNPs for interaction models presented any association with the exposure, and this finding suggests that the negative IQ association with the exposure occurred only among subjects carrying the particular alleles. Such interaction could increase the association of exposure with adverse changes of the outcome, although not observed due to confounding in the overall population (59).

Assessment of methylmercury neurotoxicity carried out by regulatory agencies relies on average association coefficients in population studies. Thus, in the Faroese birth cohort study that was used as a basis for policy making in the United States, each doubling of the prenatal methylmercury exposure was associated with a loss of about 1.5 IQ points at age 7 years (13). In that cohort, with almost 10-fold higher cord-mercury concentrations than in the present study (19), the IQ loss for a 10-fold increased exposure would then correspond to most of the estimated 5 points. At the much lower exposures in the ALSPAC cohort, no overall adverse association with IQ was detected, and the rs1042838 wild type even showed an association of an approximate IQ increase of 7 points with a 10-fold mercury increase, likely due to residual confounding from beneficial factors associated with seafood intake and perhaps advantageous social factors related to higher fish intake. Conversely, the *PROGINS* genotype was associated with a loss of 7 IQ points for a 10-fold increased exposure (i.e., a total difference of 14 points) as compared to the wild type. Assuming a similar frequency of the *PROGINS* variant at about 30% in the Faroes, most of the estimated 5-IQ-point average loss in the Faroese cohort could potentially be explained by disproportionate IQ losses in *PROGINS* carriers.

Overall, these findings emphasize that evaluation of methylmercury neurotoxicity must take into consideration the impact of genetic predisposition. While the genes identified relate to functions that are probably not directly associated with methylmercury neurotoxicity, the *PGR* heterogeneity could be of wider relevance to developmental neurotoxicity and therefore deserves further exploration.

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