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. Log_{10}-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) (β = −11.8, 95% confidence interval: −23.0, −0.6; P for interaction = 0.004) and weakly for rs662 (paraoxonase 1) (β = −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 database 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) sequenom 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 HapMap 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 \(\text{(WISC-III)}\) \((25)\), 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...
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 (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 $\log_{10}$.
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, $PON1$ rs662, $BDNF$ rs2049046, and $PGK$ 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 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 μg/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* | Model 2* | Model 3* |
| No.                             | $\beta$ | 95% CI  | No.     | $\beta$ | 95% CI  | No.     | $\beta$ | 95% CI  |
| Extended sample (new group)     | 1,051   |          | 795     |          | 792     |          | 2,281   |          | 813     |          | 1,808   |          |
| 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.

* 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.

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

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

* $\beta$ and 95% CI for $\log_{10}$(cord-mercury (ng/g)).
observed in the results after inclusion of these covariate variables. Due to a high degree of missingness of these new covariates, 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. PON1 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 rs890182, 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
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>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Major/Minor Allele</th>
<th>Cord-Mercury Concentration (ng/g)</th>
<th>P Valuea</th>
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<td></td>
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<td>Homozygous for the Major Allele</td>
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<td></td>
<td>No.</td>
<td>Mean (SD)</td>
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<td>Homozygous for the Minor Allele</td>
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Pilot SNPs (Discovery From Pilot Sample Analyses)


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<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Major/Minor Allele</th>
<th>Cord-Mercury Concentration (ng/g)</th>
<th>P Valuea</th>
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<td>Homozygous for the Major Allele</td>
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<td>No.</td>
<td>Mean (SD)</td>
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New SNPs (Discovery From Joint Sample Analyses)

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.

Table 4. Adjusted Regression Coefficientsa 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Log10(Hg), Pilot Sampleb</th>
<th>Log10(Hg), Extended Sample</th>
<th>Log10(Hg), Joint Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>95% CI</td>
<td>P for Interaction</td>
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<td></td>
<td></td>
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</tbody>
</table>

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).
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 PON1 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 PON1 rs662 association was weakly replicated with a dominant model in the joint sample analyses. Other PON1 SNPs conferring predisposition to methylmercury neurotoxicity have also been reported (48). PON1 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 PON1 (49). Because BDNF SNP (rs2049046) and TF SNP (rs3811647) associations could not be replicated in the joint

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Log&lt;sub&gt;10&lt;/sub&gt;(Cord-Slice Hg (ng/g))</th>
<th>No.</th>
<th>β</th>
<th>95% CI</th>
<th>P for Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs5746136 (SOD2) 11</td>
<td></td>
<td>797</td>
<td>0.9</td>
<td>-4.5, 6.3</td>
<td>0.082</td>
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<tr>
<td>rs5746136 (SOD2) 12+22&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>926</td>
<td>5.5</td>
<td>-0.2, 11.2</td>
<td>0.049</td>
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<tr>
<td>rs4149268 (ABCA1) 11</td>
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<td>695</td>
<td>-4.4</td>
<td>-10.7, 1.8</td>
<td>0.049</td>
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<td>764</td>
<td>6.2</td>
<td>-0.4, 12.0</td>
<td>0.049</td>
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<tr>
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<td>264</td>
<td>9.2</td>
<td>-1.6, 19.9</td>
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<td>1,257</td>
<td>1.2</td>
<td>-3.4, 5.8</td>
<td>0.038</td>
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<td>rs3890182 (ABCA1) 12+22&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>8.4</td>
<td>-0.8, 16.1</td>
<td>0.038</td>
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<td>rs2270836 (MT1M) 11</td>
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<td>281</td>
<td>10.3</td>
<td>-0.1, 20.8</td>
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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.

<sup>a</sup> 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).

<sup>b</sup> The alleles 12 and 22 were combined into a unique category due to low number of observations (22 alleles <10% of the total sample).
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-methylmercury 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 Faroese, 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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