Web Material

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

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Web Table 1: Characteristics of the Selected Confounders by Cord Hg Exposure and Child 8-year-old Total IQ (WISC-III) in a Sub-sample (combined group) of the ALSPAC Cohort (1991-2000).

Confounders of interest	**No.**	**Cord Hg Slices (ng/g) Median (IQR)**	**Total IQ[c] Mean (SD)**
Sex			
Boy	1161	23 (17-31)	105 (17)
Girl	1121	22 (17-30)	104 (15)
Maternal Age			
<30 years	1216	21 (16-28)[b]	103 (16)[b]
30 years and older	1054	24 (18-33)[b]	107 (16)[b]
Maternal education[a]			
Low	453	20 (15-26)[b]	96 (14)[b]
Middle	779	21 (17-29)[b]	103 (15)[b]
High	981	26 (19-34)[b]	111 (16)[b]
Maternal Social Class			
I-II	824	26 (19-33)[b]	111 (15)[b]
III (non-manual)	805	21 (17-29)[b]	103 (15)[b]
III (manual) IV & V	281	20 (15-27)[b]	98 (15)[b]
Housing			
Mortgaged/owned	1896	23 (17-31)[b]	106 (16)[b]
Council	153	19 (15-24)[b]	94 (15)[b]
Other	169	20 (16-28)[b]	102 (16)[b]
Parity			
0	1054	23 (17-32)[b]	106 (16)[b]
1	762	22 (17-30)[b]	105 (16)[b]
2+	392	21 (16-29)[b]	101 (16)[b]
Smoking during pregnancy			
No	1730	23 (17-31)[b]	106 (16)[b]
Yes	505	20 (16-28)[b]	101 (16)[b]
Daily Omega-3 Fatty Acid Intake from seafood 32 Week of Pregnancy			
Low (<0.05 g)	543	17 (12-22)[b]	102 (15)[b]
Moderate (≤0.12 g)	707	22 (17-29)[b]	106 (16)[b]
High (>0.12 g)	903	27 (21-36)[b]	107 (16)[b]
Selenium during pregnancy			

≤ 111 (ug/l)	472	20 (15-27)[b]	104 (16)[b]
111 (ug/l)	473	26 (19-34)[b]	107 (16)[b]
Lead during pregnancy			
≤ 3.5 (ug/l)	469	22 (17-30)	105 (16)
> 3.5 (ug/l)	474	24 (17-31)	106 (16)
Vitamin D Total during pregnancy			
≤ 64 (nmol/l)	731	22 (17-30)	104 (16)
> 64 (nmol/l)	731	23 (17-31)	105 (16)
Cotinine during pregnancy			
≤ 27 (ng/ml)	528	24 (18-32)[b]	107 (16)[b]
> 27 (ng/ml)	502	22 (16-30)[b]	103 (16)[b]

[a]Similar results for paternal education (data not shown). [b] p-value ≤ 0.05.

Web Table 2. Adjusted Regression Coefficients (β) for Log_{10} (Cord-Hg concentration (ng/g)) as a Predictor of WISC-III Outcomes Stratified by Maternal Social Class (Joint Sample of ALSPAC, 1991-2000).

	Maternal social class			
WISC-III scores	**I-II**	**III (non-man.)**	**III (man.) & IV-V**	**P-**
	β (95 % CI)	**β (95 % CI)**	**β (95 % CI)**	**interaction**
Joint sample	(n = 797)	(n = 756)	(n = 255)	
Verbal IQ	2.7 (-3.1, 8.5)	5.7 (-0.3, 11.7)	-1.4 (-12.8, 10.1)	0.620
Performance IQ	-3.4 (-9.6, 2.8)	7.7 (1.4, 13.9)	4.7 (-8.2, 17.6)	0.014
Total IQ	0.1 (-5.6, 5.7)	7.2 (1.4, 13.0)	1.7 (-9.5, 13.0)	0.196

All multivariable linear regression models adjusted for: Sex, age and examiner, parental education level, maternal age, smoking during pregnancy, parity, house ownership status and estimated omega-3 intake (from seafood' to 'omega-3 intake').

Web Table 3. Adjusted Regression Coefficients (β) for the Cord-Hg Concentration as Predictor of the 8-year WISC-III Outcomes in the Combined Group. Sensitivity Analyses with Maternal Selenium, Lead, Vitamin D and Cotinine Concentration during Pregnancy. Models corrected by IPW (ALSPAC, 1991-2000).

WISC-III scores	**Estimate (β) and 95% CI for Log_{10} (Cord-Hg (ng/g))**	
	Completed Case β (95 % CI)	**IPW Correction[f] β (95 % CI)**
	Model 2[b]	**Model 2[b]**
Selenium (Median = 111 ug/l)	n = 760	n = 760
Total IQ	-0.3 (-6.2, 5.6)	-0.2 (-5.7, 5.2)
Verbal IQ	0.1 (-6.1, 6.1)	-0.1 (-6.0, 5.9)
Performance IQ	-1.1 (-7.7, 5.5)	-0.8 (-7.1, 5.3)
	Model 3[c]	**Model 3[c]**
Lead (Median = 3 ug/l)	n = 758	n = 758
Total IQ	-1.0 (-6.8, 4.9)	-0.9 (-6.4, 4.6)
Verbal IQ	-1.1 (-7.1, 4.9)	-1.1 (-7.1, 4.9)
Performance IQ	-0.9 (-7.4, 5.6)	-0.8 (-7.0, 5.4)
	Model 4[d]	**Model 4[d]**
Vitamin D Total (Median = 64 nmol/l)	n = 1178	n = 1178
Total IQ	1.0 (-3.7, 5.6)	0.7 (-3.9, 5.3)
Verbal IQ	1.4 (-3.3, 6.1)	1.0 (-3.9, 5.9)
Performance IQ	0.5 (-4.6, 5.6)	0.4 (-4.6, 5.4)
	Model 5[e]	**Model 5[e]**
Cotinine (Median = 27 ng/ml)	n = 830	n = 830
Total IQ	-1.5 (-7.0, 4.0)	-1.7 (-7.4, 4.0)
Verbal IQ	-1.9 (-7.6, 3.8)	-1.5 (-7.6, 4.5)
Performance IQ	-0.7 (-7.0, 5.6)	-1.3 (-7.7, 5.1)

[a]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, estimated omega-3 intake (from seafood' to 'omega-3 intake'). [b]Additionally adjusted for maternal selenium blood concentration during pregnancy. [c]Additionally adjusted for maternal lead blood concentration during pregnancy. [d]Additionally adjusted for maternal total vitamin D blood concentration during pregnancy. [e]Additionally adjusted for maternal cotinine urine concentration during pregnancy. [f]Coefficients estimated using IPW. Weights = inverse of the probability of being a complete case. Predicted probabilities were estimated by using logistic regression with age, parental education level, maternal smoking during pregnancy, social class, parity and house ownership status, estimated omega-3 intake (from seafood' to 'omega-3 intake') as predictors of missingness.

Web Table 4. Adjusted Regression Coefficients (β) for the Cord-Hg Concentration (ng/g) as Predictor of WISC-III Total IQ by Selected Genotypes (Joint Sample of ALSPAC corrected by IPW[b]; 1991-2000).

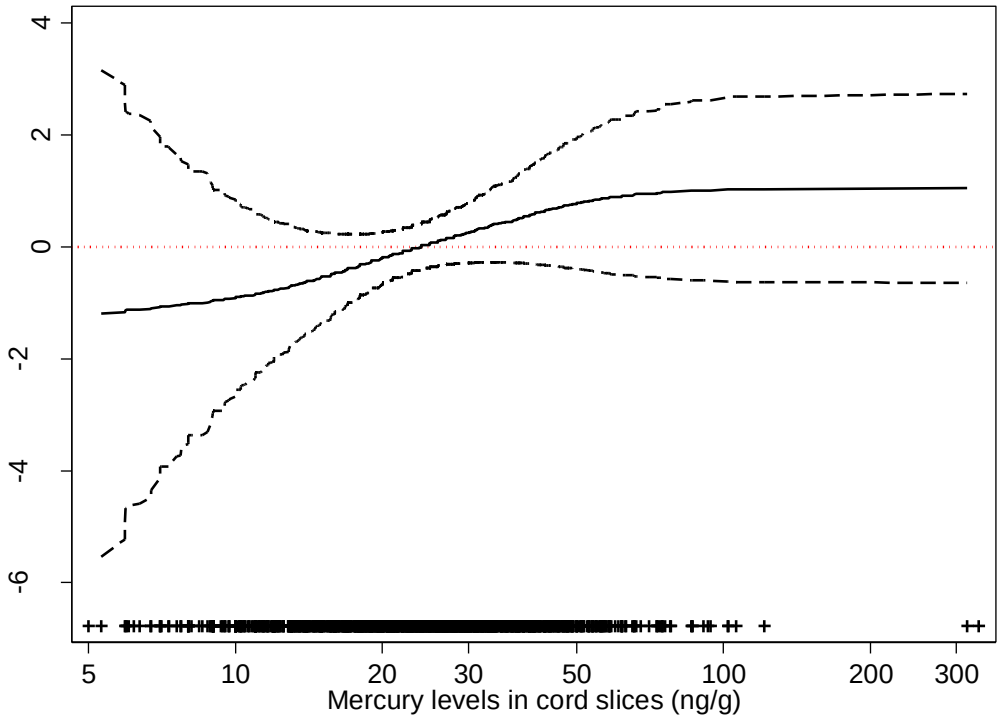
WISC-III scores		**Log_{10} (Cord Hg Slices (ng/g))**		
Joint Sample	**n**	**Estimate (β)[b]**	**95 % CI**	**P-interaction**
Total IQ	1723			
Pilot Study SNPs (Re-Tested with the Joint Sample)				
rs3811647 (*TF*) 11	760	5.9	0.3, 11.5	
rs3811647 (*TF*) 12	768	0.7	-5.5, 7.0	0.683
rs3811647 (*TF*) 22	195	0.7	-10.2, 11.6	
rs662 (*PON1*) 11	898	7.6	1.8, 13.4	0.056
rs662 (*PON1*) 12+22[a]	825	-2.5	-7.8, 2.8	
rs1042838 (*PGR*) 11	1197	7.1	2.5, 11.6	0.001
rs1042838 (*PGR*) 12+22[a]	526	-7.6	-15.0, -0.2	
rs2049046 (*BDNF*) 11	514	4.3	-3.4, 12.0	
rs2049046 (*BDNF*) 12	854	3.2	-2.2, 8.7	0.999
rs2049046 (*BDNF*) 22	355	1.3	-6.6, 9.1	
New SNPs (Discovery from Joint Sample Analyses)				
rs5746136 (*SOD2*) 11	797	0.4	-5.2, 6.0	0.057
rs5746136 (*SOD2*) 12+22[a]	926	5.6	0.1, 11.0	
rs4149268 (*ABCA1*)11	695	-4.6	-10.7, 1.4	
rs4149268 (*ABCA1*)12	764	6.1	0.2, 11.9	0.043
rs4149268 (*ABCA1*)22	264	9.4	-2.4, 21.1	
rs3890182 (*ABCA1*)11	1257	0.8	-3.6, 5.3	0.043
rs3890182 (*ABCA1*)12+22[a]	466	8.6	0.2, 17.0	
rs2270836 (*MT1M*)11	645	-1.8	-8.4, 4.7	
rs2270836 (*MT1M*)12	777	5.3	-0.3, 10.8	0.048
rs2270836 (*MT1M*)22	281	10.3	-0.2, 20.8	

TF (Transferrin); *PON1* (Paraoxonase 1); *BDNF* (Brain-Derived Neurotrophic Factor); *PGR* (Progesterone Receptor); *SOD2* (superoxide dismutase 2); *ABCA1* (ATP binding cassette subfamily A member 1); *MT1M* (metallothionein 1M). All multivariable linear regression models adjusted for: Sex, age and examiner, parental education level, maternal age, smoking during pregnancy, social class, parity and house ownership status, estimated omega-3 intake (from seafood' to 'omega-3 intake'). [a]The alleles 12 and 22 were combined into a unique category due to low number of observations (22 alleles < 10 % of the total sample). [b]Coefficients estimated using IPW. Weights = inverse of the probability of being a complete case. Predicted probabilities were estimated by using logistic regression with age, parental education level, maternal smoking during pregnancy, social class, parity and house ownership status, estimated omega-3 intake (from seafood' to 'omega-3 intake') as predictors of missingness.

Web Table 5. Adjusted Regression Coefficients (β) for the Cord-Hg Concentration as Predictor of the 8-year WISC-III Outcomes in the Combined Group, General and by *PGR* rs1042838 variants. Main Models and Additional Adjustments for Maternal Healthy Diet Score and Child Processed Diet Score (ALSPAC, 1991-2000).

	Umbilical Cord Mercury	
WISC-III scores	**Main Model [a]**	**Additional Adjusment Model [b]**
	β (95 % CI)	**β (95 % CI)**
Joint sample		
Total IQ (n = 1564)	1.4 (-2.5, 5.3)	1.2 (-2.7, 5.1)
Total IQ by rs1042838 (*PGR*) 11 (n = 1034)	5.4 (0.5, 10.4)	5.2 (0.3, 10.2)
Total IQ by rs1042838 (*PGR*) 12 + 22[c] (n = 458)	-7.6 (-15.1, -0.1)	-7.5 (-14.9, -0.1)
p-for-interaction	0.001	0.001

[a] Multivariable linear regression models adjusted for: Sex, age and examiner, parental education level, maternal age, smoking during pregnancy, social class, parity, house ownership status and estimated omega-3 intake (from seafood' to 'omega-3 intake').
[b] Multivariable linear regression models additionally adjusted for maternal healthy diet score during pregnancy and child processed diet score.
[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).



Web Figure 1. GAM plot for WISC-III Total IQ showing the association with Mercury levels in Cord slices. Dash lines are 95% CI. Tick marks on the x-axis display de distribution of observed Hg concentrations. Analysis adjusted by sex, age and examiner, parental education level, maternal age, smoking during pregnancy, social class, parity and house ownership status, estimated omega-3 intake (from seafood' to 'omega-3 intake'). The alleles 12 and 22 were combined into a unique category due to low number of observations (22 alleles < 10 % of the total sample). ALSPAC, 1991-2000.