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KEAP1 polymorphisms and neurodevelopmental outcomes in children with exposure to prenatal MeHg from the Seychelles Child Development Study Nutrition Cohort 2

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Abstract

Background—Humans differ in the metabolism of the neurotoxicant methyl mercury (MeHg). This variation may be partially due to variation in genes encoding the transcription factor Nuclear factor E2-related factor 2 (NRF2) and its negative regulator Kelch-like ECH-Associated Protein 1

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

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(KEAP1), which regulate glutathione and related transporter and antioxidant proteins that play a role in the metabolism and neurotoxicity of MeHg.

Aim—To elucidate a potential risk from genetic variation in *NFE2L2 (encoding NRF2)* and *KEAP1* toward prenatal mercury exposure and child neurodevelopmental outcomes at 20 months and 7 years of age in a population with variable prenatal exposure to MeHg from maternal fish consumption.

Material and Methods—Nutrition Cohort 2 is a mother–child cohort in the Republic of Seychelles. Children were genotyped for *NFE2L2* (rs2364723, rs13001694) and *KEAP1* (rs8113472, rs9676881) polymorphisms (N = 1,285 after removing siblings). Total mercury (Hg) was measured in cord blood as a biomarker for prenatal MeHg exposure. Child neurodevelopmental outcomes included the Bayley Scales of Infant Development II administered at 20 months of age, and outcomes across multiple neurodevelopmental domains from 14 tests administered in children and 3 instruments completed by parents when children were 7 years of age.

Results—The mean cord blood MeHg concentration was 34 (95% CI 11, 75) μg/L. None of the four polymorphisms had a significant association ($p<0.05$) with either cord MeHg or neurodevelopmental test results at 20 months. There were no significant associations between either *NFE2L2* polymorphism and any developmental test scores. At 7 years, children carrying *KEAP1* rs8113472 CA showed significantly worse performance on psychomotor function than children with the CC variant (finger tapping, dominant hand: β −1.19, SE 0.34; finger tapping, non-dominant hand: β −0.92, SE 0.31) and worse social communication (SCQ Total: β 0.65, SE 0.27). Children carrying rs8113472 AA, versus children with CC, showed significantly better performance on social communication (SRS Total: β −8.88, SE 3.60). Children carrying *KEAP1* rs9676881 AG, versus children with GG, showed significantly worse performance on psychomotor function (trailmaking A time: β 8.66, SE 3.37) and cognition (KBIT Matrices: β −0.96, SE 0.36).

Conclusion—No associations between *NFE2L2* and *KEAP1* polymorphisms and MeHg concentration were identified. However, at 7 years, *KEAP1* polymorphisms were associated with differences in neurodevelopmental outcomes in children from a population with high fish intake.

Keywords

child neurodevelopment; cord mercury; *KEAP1* ; *NFE2L2* ; *NRF2*

1. Introduction

Methyl mercury (MeHg) is present in all fish in different concentrations. High levels of MeHg have detrimental effects on the nervous system (Clarkson et al. 2003, Johansson et al. 2007). Methylmercury can cross the placenta and blood–brain barrier and present a risk for the developing fetus (Costa et al. 2004, Johansson et al. 2007). However, the MeHg concentrations at which the fetal brain is affected remain unclear. Studies have evaluated associations between prenatal MeHg exposure and child neurodevelopment in different populations, but the results have not been consistent. Some studies reported adverse associations with prenatal MeHg and child neurodevelopment (Grandjean et al. 1997, Oken et al. 2005, Sagiv et al. 2012, Vejrup et al. 2016), whereas others ranged from no association

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to a moderate beneficial association (Davidson et al. 1998, Myers et al. 2003, Daniels et al. 2004, Hibbeln 2007, Llop et al. 2012, Strain et al. 2015).

MeHg undergoes complex dynamics in mammalian cells, suggesting that differences in cellular metabolism might influence MeHg toxicity. Binding of MeHg to the oxidative stress response factor glutathione (GSH) is an important pathway for MeHg kinetics, which may be mediated by glutathione *S*-transferases (GSTs) (Broberg et al. 2004, Vorojeikina et al. 2017). ATP-binding cassette (ABC) transporters can then eliminate GSH-MeHg conjugates from the cells, facilitating their excretion (Fujimura and Usuki 2020). This MeHg excretion mechanism is therefore under the influence of glutamate-cysteine ligase (GCL), which is the rate-limiting enzyme for biosynthesis of GSH.

Nuclear factor E2-related factor 2 (NRF2) and its modulator Kelch-like ECH-Associated Protein 1 (KEAP1), also called the NRF2-KEAP1 response pathway, directly modulate a number of oxidative stress response factors, including GCL, and are therefore thought to be global regulators of MeHg toxicokinetics. The NRF2–KEAP1 response pathway can be activated by the direct interaction of MeHg with KEAP1 (Wang et al. 2009, Culbreth et al. 2017, Gunderson et al. 2020). Following its interaction with MeHg, KEAP1 dissociates from NRF2, which is then translocated to the nucleus where it induces the expression of oxidative stress response genes such as *GCLM* and *GCLC* by binding to the antioxidant response element (Fujimura and Usuki 2020, Gunderson et al, 2020). This pathway also regulates MeHg toxicodynamics, for example, functional assays in *Drosophila melanogaster* (fruit fly) have demonstrated that enhanced NRF2 activity has a protective effect when embryos are exposed to MeHg during embryonic development (Rand et al. 2009, Gunderson et al. 2020).

Accordingly, polymorphisms in any of the GSH-related genes may influence MeHg elimination and the toxic effects in the body. Examination of these effects in humans requires a large, well-characterized study population. The Seychelles Child Development Study Nutrition Cohort 2 (SCDS NC2) is a longitudinal observational mother–child cohort from the Republic of Seychelles that exhibits high fish consumption rates (Strain et al. 2015). In previous studies in the SCDS NC2 cohort, maternal genetic variation in genes involved in GSH synthesis (Wahlberg et al. 2018) and maternal and child genetic variation in ABC transporters (Engström et al. 2016, Love et al. 2022) were associated with MeHg concentrations in maternal hair and cord blood. The observed associations also suggested that maternal GSH and child ABC genetics may modify associations between MeHg exposure and neurodevelopmental outcomes in 20-month-old children (Wahlberg et al. 2018, Love et al. 2022). However, little is known about the function of the upstream regulatory genes *NFE2L2* (encoding NRF2) and *KEAP1* in moderating MeHg metabolism and toxicity.

In this study, we assessed whether polymorphisms in *NFE2L2* and *KEAP1* influence prenatal MeHg exposure and child neurodevelopment outcomes at 20 months and 7 years of age. Our hypothesis is that genetic variation in *NFE2L2* and *KEAP1* may interfere with activation of the NRF2–KEAP1 response pathway, MeHg elimination, and toxicity during gestation, and thereby lead to higher cord MeHg concentrations and increased susceptibility to adverse neurodevelopmental outcomes in childhood if they are present at the exposure levels we studied.

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2. Materials and Methods

2.1 Study population

The study participants belong to the Seychelles Child Development Study (SCDS) Nutrition Cohort 2 (NC2). This study aims to investigate pregnant women's exposure to MeHg, its association with child neurodevelopment, and whether this association is influenced by nutrition and genetics. From 2008 to 2011, 1,535 pregnant mothers were recruited at their first antenatal visit (from 14 weeks of gestation) at eight health centres across the island of Mahé, Republic of Seychelles (Strain et al. 2015). The participants had to meet certain criteria, such as originating from Seychelles and being ≥16 years of age. Exclusions for subsequent analyses were prespecified in each analysis plan; children were excluded if they were twins, or if they had maternal prenatal complications, birthweight <1600g, or death, head trauma, seizures, or disability before examination. When infants were about 20 months old (range 15.9 to 32.6 months), 1,458 (95.0%) were eligible to be called up for neurodevelopmental evaluation as they had no complicating health conditions reported (Strain et al. 2015). From 2015 to 2018, 1,441 children (93.9%) were eligible for a 7-year evaluation (range 7.0 to 7.9 years); saliva samples were collected for DNA extraction, and a battery of neurodevelopmental tests were performed (Strain et al. 2021). The study was conducted according to the guidelines of the Declaration of Helsinki, and all study procedures involving participants were reviewed and approved by the Seychelles Ethics Board, the Research Subjects Review Board at the University of Rochester, and the Regional Ethics Committee at Lund University, Sweden.

2.2 Cord MeHg analyses

We used cord blood MeHg as reflective of prenatal MeHg exposure since it is more proximate to the child than maternal hair, which in turn is relevant since we are evaluating the role of the child genotype. Total Hg in cord whole blood was determined for 1,058 children (68.9%) using Cold Vapor Atomic Absorption Spectroscopy (CVAAS) and a Laboratory Data Control Mercury Monitor Model #1235 as previously described (Magos and Clarkson, 1972, Cernichiari et al., 1995). The limit of detection (LOD) was 1.75 μg Hg/L. Total Hg was presumed to be primarily MeHg, as greater than 80% of total Hg in blood from fish consumers is reported to be MeHg (National Research Council, 2000; Sherlock et al., 1984; Phelps et al., 1980). Certified mercury standards (Fisher SM114-100 and Ricca Chemical Company AHG1KN-100) and certified reference material (Seronorm™, Sero) were utilized for internal quality control. For purposes of external quality control, the laboratory participated in the Interlaboratory Comparison Program for blood sponsored by the Center of Toxicology of Quebec (INSPQ), Canada.

2.3 Neurodevelopmental assessment

At 20 months (range 15.9 to 32.6 months), 1,458 infants (95.0% of enrolled) were eligible and developmental testing was conducted on 1,394 infants (95.6% of eligible) with the Bayley Scales of Infant Development (BSID-II), amongst other tests (Strain et al. 2015). Testing was conducted by specially trained nurses at the Child Development Centre, Mahé as previously described (Strain et al 2015). The two scores from the BSID-II, the Mental Development Index (MDI) and Psychomotor Development Index (PDI), were prespecified

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for this toxicodynamic analysis because we previously reported an association between prenatal MeHg and PDI, but only after considering effect modification by polyunsaturated fatty acids (Strain et al. 2015).

At 7 years (range 7.0 to 7.9 years), 1,441 children (93.9% of enrolled) were eligible and 1,401 children (97.2% of eligible) completed neurodevelopmental testing conducted by specially trained nurses at the Child Development Centre, Mahé (Strain et al. 2021). The children's test battery included 14 primary neurodevelopmental endpoints covering the following domains:

- Language ability: Boston Naming Test (BNT) and the Clinical Evaluation of Language Fundamentals, Fifth Edition (CELF; six endpoints: Following Directions - FD, Linguistic Concepts - LC, Recalling Sentences - RS, Sentence Comprehension - SC, Understanding Spoken Paragraphs - USP, and Total);
- Cognition: Kaufman Brief Intelligence Test, Second Edition (KBIT; two endpoints: Word Knowledge - WK, and Matrices - MC);
- Psychomotor development: Finger Tapping (FT; two endpoints: Dominant Hand - DH, and Nondominant Hand - NDH) and Trailmaking A (TM A Time)
- Scholastic Achievement: Woodcock-Johnson Test of Achievement, Second Edition (WJ; two endpoints: Applied Problems - AP, and Letter Word - LW).

Parents were asked to complete the following instruments for an additional 3 primary endpoints: the Child Behaviour Check List (CBCL; Total Score) assessed behavioral problems and social competencies, whereas the Social Responsiveness Scale (SRS) and the Social Communication Questionnaire (SCQ) are screening instruments for autism spectrum disorder.

2.4 Child genetic analyses

At 7 years, 1,311 children (85.4% of enrolled) provided saliva samples that were collected in 15-mL polystyrene tubes (Sarstedt, Nümbrecht, Germany). Approximately 1 mL of BL Buffer (Omega Bio-tek, Norcross, USA) was added for preservation, and the samples were stored at −80 °C. The samples were shipped to Lund University, Sweden in Crēdo boxes (Pelican BioThermal, Plymouth, MN, USA) at regular intervals from April 2016 to November 2018 and stored at −20 °C upon arrival. For DNA extraction, the Omega Bio-tek kit (Omega Bio-tek, Norcross, USA) was used following the manufacturer's instructions. The DNA was eluted with 100 μL elution buffer and stored at −20 °C for further analyses. All the single-nucleotide polymorphism (SNP) custom genotyping assays were acquired from Thermo Fisher Scientific (Waltham, MA, USA). Two SNPs per gene were selected based on minor allele frequency >10%, potential functional impact based on the literature, and no linkage between them. Genotyping of *NFE2L2* rs2364723 and rs13001694 (TaqMan assays ID C_351878_10 and C_31613510_10) and *KEAP1* rs8113472 and rs9676881 (TaqMan assays ID C_9323048_10 and C_9323015_10) was performed by TaqMan real-time PCR using an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Thermo Fisher), following the company's standard conditions. After all samples were analysed, >5% of the samples were selected and re-analysed for all SNPs to verify genotyping data,

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and 100% concordance was found among the duplicates. To examine data quality, the Hardy-Weinberg equilibrium was evaluated using a Chi-Square test.

2.5 Statistical analyses

Linear models were used for toxicokinetic analysis to estimate the association of children's SNPs with cord blood MeHg. The mean cord blood MeHg for children with each genotype are presented along with their 95% confidence intervals. In toxicodynamic analyses, we used linear regression models to estimate the association of SNPs with neurodevelopmental test scores. The homozygote with the most subjects in our sample is the reference category and the mean difference in outcome associated with other genotypes is presented with its standard error. When the outcome means differ significantly across genotype levels based on a 2 df test, the p-value comparing each genotype to the homozygote are reported. In these toxicodynamic models, we also adjusted for factors known to be associated with child neurodevelopment and chosen *a priori* as precision variables, including child sex and age at testing, maternal age at delivery and maternal KBIT IQ score, presence of two parents in the household, and Hollingshead socioeconomic score (Strain et al. 2021). To investigate whether polymorphisms in these genes could influence the relationship between cord blood Hg concentrations and neurodevelopment, we analyzed the interaction between SNPs and cord blood Hg on each outcome. Statistical significance was assigned to p-values less than 0.05 and the Bonferroni correction for multiple testing was also reported. Statistical analyses were undertaken using R (version 3.6.2; R Core Team, 2020).

The cut-off for Bonferroni correction for the neurodevelopment tests at 20 months of age was 0.05/8=0.00625. The cut-off for Bonferroni correction for the neurodevelopment tests at 7 years of age was 0.05/76=0.000658. None of the associations remained significant after this correction.

3. Results

Background characteristics for the mothers and children at 20 months and 7 years are presented in Table 2. Of the 1,394 infants who were eligible and completed at least one test at the 20-month timepoint, 1,216 were included in the analysis and 160 excluded because there was no saliva sample for genotyping, 13 because they were second siblings in the cohort, and 11 because maternal IQ was missing. Of the 1,401 children who were eligible and completed at least one test at the 7-year timepoint, 1,236 were included in the analysis and 147 excluded because there was no saliva sample for genotyping, 13 because they were second siblings in the cohort, and 10 because maternal IQ was missing. The percent of children living in a two-parent household decreased from 73% to 50% from age 20 months to 7 years. The summaries of the neurodevelopmental tests performed at age 20 months and 7 years are presented in Supplementary Table 1.

SNP characteristics and minor allele frequencies (MAFs) for the genotypes of 1,285 NC2 children (this excludes 13 second twins and 13 other second siblings) are presented in Table 1. All SNPs were in Hardy-Weinberg equilibrium. *NFE2L2* rs13001694 and *KEAP1* rs8113472 showed a similar allele frequency to African populations, whereas *NFE2L2*

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rs2364723 and *KEAP1* rs9676881 showed a higher frequency in the Seychelles than in African populations (26% versus 14% and 42% versus 24%, respectively).

The cord MeHg ranged from 2 to 181 μg/L with a mean of 34 μg/L among the 874 children with genotypes and measured cord MeHg. Mean Hg concentrations in cord blood for each SNP genotype are presented in Table 3. Cord MeHg was not significantly associated with any of the SNPs.

Table 4 shows the associations of genotypes with neurodevelopmental outcomes. Neurodevelopmental outcomes at 20 months of age were not significantly associated with any of the SNPs. At the age of 7, neurodevelopmental outcomes were not significantly associated with either *NFE2L2* SNP.

For *KEAP1*, several associations with different test outcomes at 7 years of age were present. *KEAP1* rs8113472 was significantly associated with outcomes from psychomotor function and social communication tests in models that did not include MeHg. Children carrying the CA genotype had significantly lower scores (worse performance) for FT dominant hand and FT non-dominant hand (psychomotor function), and significantly higher scores (worse performance) for SCQ total (social communication) than children carrying the CC genotype. The same pattern appeared in the other social communication test (SRS), but in this case the CA genotype children were not significantly different. Children carrying AA showed lower scores (worse performance) for FT on both hands (psychomotor function), although the differences from CC children were not statistically significant. However, children carrying AA had significantly lower scores (better performance) in the SRS total (social communication), than children carrying CC. The same general pattern is seen with the other social communication test (SCQ), where the CA children had significantly higher scores than the CC children, while the SCQ scores of the AA children were not significantly different from CC. Children carrying *KEAP1* rs9676881 AG had significantly slower (worse) TMA times (psychomotor function) and lower (worse) KBIT Matrices scores (cognition) than children with GG (the minor homozygote). For each outcome with significant differences, the mean outcome level for each genotype and their confidence intervals are presented in Supplementary Figure 1.

Only one statistically significant interaction (p=0.013) was observed for child *KEAP1* rs8113472 genotype with cord blood Hg concentrations on the CELF USP. Children homozygous for the rare allele (genotype AA) showed higher CELF USP scores with higher cord Hg (slope=0.094, standard error=0.034), whereas for the common and heterozygous genotypes (CC and CA), CELF USP did not significantly associate with cord Hg. There are only 20 children with this rare genotype in our cohort, so this is a speculative association.

4. Discussion

In this paper, we evaluated whether polymorphisms in *NFE2L2* and *KEAP1* influence prenatal MeHg exposure and child neurodevelopment outcomes in children at 20 months and 7 years of age in a population with high fish consumption. No associations of MeHg with either *NFE2L2* or *KEAP1* were present. We did find associations between

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genotypes when MeHg was not included in the models. We found that children carrying the *KEAP1* rs9676881 GG genotype showed significantly better performance for two neurodevelopmental tests (TMA and KBIT MC) than the AG carriers.

Whereas *KEAP1* rs8113472 was not associated with cord MeHg, there were some interesting findings between genotypes. The variant A allele was associated with poorer performance in tests for cognition, psychomotor function, and social communication compared to the other alleles. The associations were strongest for the heterozygotes, which may be due to the few individuals of the variant homozygotes showing a wide variation in performance. These findings suggest that *KEAP1* rs8113472 might have an indirect role in neurodevelopment, even though we did not find it significantly related to MeHg exposure. It should be noted that the effect sizes of the genotypes were in general small, and not statistically significant after Bonferroni correction.

KEAP1 rs8113472 is an intron variant, while *KEAP1* rs9676881 is a variant situated downstream of the gene. Both SNPs are, according to the GTex portal (www.gtexportal.org/), associated with differential gene expression, but the data for *KEAP1* rs8113472 are poor due to very few individuals with the AA genotype. The *KEAP1* rs8113472 and rs9676881 A alleles show higher expression in different tissues, including the brain, which hypothetically could indicate higher *KEAP1* protein level and in turn less NRF2 activation of oxidative stress factor genes.

There was no evidence for an influence of *NFE2L2* on MeHg levels or neurodevelopmental outcomes. Even though NRF2 has been found to be protective of MeHg toxicity in a fly model (Gunderson et al. 2020, Rand et al. 2009), we could not find evidence that human NRF2 variation at the gene level modifies the elimination or response to MeHg.

Strengths of the study include the large mother–child cohort with high fish consumption and, in consequence, a relatively high MeHg exposure. Another strength is the high number of children analysed for neurodevelopment outcomes at 20 months and 7 years of age with very little loss to follow up. The study also measured and included in the analysis many confounding variables which are known to be related to neurodevelopmental test scores.

Limitations include the small number of SNPs evaluated because there may be other genetic variants with different associations with the NRF2-KEAP1 response pathway, which may in turn have a greater influence on MeHg toxicokinetics.

In conclusion, we found no evidence that prenatal MeHg exposure was associated with the SNPs we studied. However, our results suggest that children's *KEAP1* genotype may influence neurodevelopment outcomes at 7 years of age in a high fish-eating population although they were not significant after correction for multiplicity. These findings need to be verified in independent studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Polymorphisms of *NFE2L2* and *KEAP1* were not associated with cord MeHg concentration
- Having *KEAP1* rs8113472 CA was associated with worse psychomotor function
- Having *KEAP1* rs8113472 AA was associated with better social communication
- *KEAP1* rs9676881 AG was associated with worse psychomotor function and cognition

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Table 1

Information on single-nucleotide polymorphisms (SNPs) and minor allele frequencies (MAFs) measured in the Seychelles Nutrition Cohort 2 (NC2) study (n=1285 genotyped excluding 26 second siblings).

Gene	SNP (Alleles[a])	Chromosome	SNP type[b]	Functional effect[b]	MAF			
					NC2	Africa[c]	South Asia[c]	Europe[c]
NFE2L2 / NRF2	rs13001694 (A/**G**)	2	Intronic	intron variant	32	33	13	38
	rs2364723 (**C**/G)	2	Intronic	intron variant	26	14	53	31
KEAP1	rs8113472 (C/**A**)	19	Intronic	intron variant	12	19	1	10
	rs9676881 (A/**G**)	19	Downstream Variant	regulatory region variant	42	24	54	65

[a] Minor alleles in bold.

[b] According to the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

[c] According to the Ensembl Genome Browser (www.ensembl.org). Minor allele frequency (MAF) averages for African, South Asian, and European populations.

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Table 2

Background characteristics of the study population for the Nutrition Cohort 2 in the Seychelles Child Development Study.

Timepoint **Variable**	**n**	**mean**	**min, max**	**5th, 95th percentiles**
At birth				
Cord blood MeHg (μg/L)	874	34	2, 181	11, 75
20 months				
Child test age (months)	1216	21	16, 33	19, 23
Maternal age at delivery (years)	1216	27	16, 45	18, 39
Maternal KBIT (raw score)	1216	30	12, 46	17, 39
Hollingshead SES at 20 months	1216	32	11, 63	16, 50
Family status at 20 months (% with 2 parents in household)	1216	73%		
Percent girls	1216	48%		
7 years				
Child test age (years)	1236	7	7.0, 7.9	7.1, 7.7
Maternal age at delivery (years)	1236	27	16, 47	18, 39
Maternal KBIT (raw score)	1236	30	4, 46	17, 39
Hollingshead SES at 7 years	1236	33	8, 63	17, 52
Family status at 7 years (% with 2 parents in household)	1236	50%		
Percent girls	1236	48%		

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Table 3

Mean cord blood MeHg of study population ($N = 874$) and associations of genotypes with Hg concentrations for the Nutrition Cohort 2 in the Seychelles Child Development Study. No *p*-value showed significance.

SNP	Genotype	n	Cord MeHg (μg/L)	95% CI	Gene *p*-value
NFE2L2 rs2364723	GG	485	33.84	(32.01,35.68)	0.678
	CG	327	33.51	(31.28,35.75)	
	CC	62	36.02	(30.89,41.15)	
NFE2L2 rs13001694	AA	406	33.36	(31.36,35.36)	0.237
	AG	382	35.02	(32.95,37.08)	
	GG	86	31.21	(26.85,35.56)	
KEAP1 rs8113472	CC	664	33.72	(32.15,35.29)	0.923
	CA	190	34.30	(31.37,37.23)	
	AA	20	34.83	(25.79,43.87)	
KEAP1 rs9676881	AA	304	31.93	(29.62,34.24)	0.098
	AG	409	35.28	(33.29,37.28)	
	GG	161	33.96	(30.78,37.14)	

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Table 4

Adjusted mean difference from reference in neurodevelopmental outcomes at 20 months and 7 years (N = 1,141–1,236) between children with different SNPs in NFE2L2 or KEAP1 genes[a]. The homozygote with the most subjects in our sample is the reference category and the mean difference in outcome (β) associated with changing the genotype is given with its standard error (SE). For each model, the first row gives the p-value for the 2df test for a change in outcome by genotype, conditional on the other covariates. When the outcome means differ significantly across genotype levels based on a 2 df test (Gene p-value), the p-value for the mean differences from the homozygote are given (p).

Response	Gene *p*-value	*NFE2L2* rs2364723		Gene *p*-value	*NFE2L2* rs13001694		Gene *p*-value	*KEAP1* rs8113472		Gene *p*-value	*KEAP1* rs9676881	
		CG	CC		AG	GG		CA	AA		AG	GG
Outcomes at 20 months												
MDI	0.513			0.512			0.832			0.218		
β		0.16	0.63		−0.20	−0.53		−0.11	0.55		0.01	0.66
SE		0.31	0.56		0.31	2.44		0.36	1.09		0.32	0.42
PDI	0.529			0.880			0.829			0.601		
β		−0.15	0.13		0.08	0.02		0.01	−0.35		0.14	−0.03
SE		0.16	0.30		0.16	0.26		0.19	0.58		0.17	0.22
Outcomes at 7 years												
TM A Time	0.251			0.766			0.905			**0.030**		
β		3.89	−1.76		−1.76	0.28		−1.18	−2.33		4.19	−4.47
SE		2.61	4.69		2.61	4.19		3.06	9.18		2.75	3.54
p											0.127	0.207
BNT Total	0.858			0.933			0.063			0.288		
β		0.04	0.29		−0.04	−0.17		−0.63	1.39		0.19	0.63
SE		0.29	0.53		0.29	0.46		0.34	1.04		0.31	0.40
CELF Total	0.761			0.650			0.258			0.168		
β		0.55	1.03		−0.76	0.37		−1.61	2.45		−0.14	2.18
SE		0.97	1.74		0.97	1.53		1.12	3.43		1.02	1.33
CELF FD	0.411			0.900			0.143			0.628		
β		0.31	0.00		−0.01	−0.17		−0.35	1.27		0.23	0.25
SE		0.24	0.44		0.24	0.38		0.28	0.87		0.26	0.33
CELF LC	0.932			0.457			0.967			0.922		
β		0.05	0.10		−0.22	−0.06		−0.05	0.00		−0.07	−0.08
SE		0.18	0.32		0.18	0.28		0.21	0.63		0.19	0.24
CELF RS	0.996			0.704			0.445			0.107		
β		0.03	0.07		−0.06	0.54		−0.67	−0.14		−0.09	1.13
SE		0.46	0.82		0.46	0.72		0.53	1.62		0.48	0.63
CELF SC	0.204			0.666			0.070			0.247		
β		−0.14	0.68		−0.13	0.21		−0.52	1.14		−0.32	0.16
SE		0.25	0.45		0.25	0.39		0.29	0.88		0.26	0.34

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Response	Gene *p*-value	*NFE2L2* rs2364723		Gene *p*-value	*NFE2L2* rs13001694		Gene *p*-value	*KEAP1* rs8113472		Gene *p*-value	*KEAP1* rs9676881	
		CG	CC		AG	GG		CA	AA		AG	GG
CELF USP	0.668			0.186			0.759			0.524		
β		0.07	0.32		−0.36	−0.22		0.09	0.47		0.01	0.28
SE		0.20	0.36		0.20	0.31		0.23	0.71		0.21	0.27
CBCL Total	0.982			0.193			0.056			0.958		
β		0.23	−0.13		−1.71	1.87		1.77	−10.12		−0.02	0.48
SE		1.36	2.46		1.37	2.14		1.58	4.95		1.44	1.88
KBIT WK	0.590			0.364			0.228			0.442		
β		0.02	−0.83		−0.45	0.52		−0.07	−2.87		−0.63	−0.35
SE		0.47	0.84		0.47	0.74		0.54	1.67		0.49	0.64
KBIT MC	0.402			0.264			0.073			**0.029**		
β		0.33	−0.83		0.03	−0.67		−0.73	−0.54		−0.35	0.62
SE		0.28	0.84		0.28	0.44		0.32	1.00		0.29	0.38
p											0.238	0.108
WJ AP	0.117			0.794			0.870			0.403		
β		−0.02	0.74		−0.07	−0.22		−0.11	−0.20		−0.22	0.10
SE		0.21	0.37		0.21	0.33		0.24	0.74		0.22	0.28
WJ LW	0.834			0.816			0.151			0.911		
β		−0.22	1.25		−0.44	−1.29		0.10	9.16		−0.54	0.00
SE		1.32	2.38		1.32	2.09		1.53	4.71		1.39	1.82
FTDH	0.308			0.103			**0.002**			0.132		
β		0.31	−0.45		−0.41	0.53		**−1.19**	−1.01		0.31	−0.46
SE		0.30	0.53		0.30	0.47		**0.34**	1.05		0.31	0.41
p								**0.001**	0.340			
FTNDH	0.999			0.606			**0.011**			0.794		
β		−0.00	0.02		−0.15	0.26		**−0.92**	−0.59		−0.03	−0.24
SE		0.27	0.48		0.27	0.42		**0.31**	0.94		0.28	0.36
p								**0.003**	0.529			
SCQ Total	0.801			0.705			**0.008**			0.643		
β		−0.11	−0.24		−0.18	0.02		**0.65**	−1.58		−0.08	−0.31
SE		0.24	0.43		0.24	0.37		**0.27**	0.86		0.25	0.33
p								**0.017**	0.068			
SRS Total	0.635			0.708			**0.026**			0.611		
β		−0.72	−0.24		−0.47	0.83		1.11	**−8.88**		0.96	1.08
SE		1.01	0.43		1.01	1.59		1.17	**3.60**		1.07	1.38
p								0.346	**0.014**			

Abbreviations: *N* = Number of individuals, MDI = Mental Development Index, PDI = Motor Development Index, TM = Trailmaking, BNT = Boston Naming Test, CELF = Clinical Evaluation of Language Fundamentals, FD = Following Directions, LC = Linguistic Concepts, RS = Recalling Sentences, SC = Sentence Comprehension, USP = Understanding Spoken Paragraphs, CBCL = Child Behavior Check List, KBIT = Kaufman Brief Intelligence Test, WK = Word Knowledge Sum, MC = Matrices, WJ = Woodcock-Johnson, FTDH = Finger Tapping Dominant Hand, FTNDH = Finger Tapping Non-Dominant Hand, SCQ = Social Communication Questionnaire, SRS = Social Responsiveness Scale.

Bonferroni correction threshold 0.05/76=0.000658; no significant results after correction.

[a]These models are adjusted for child sex and age at testing, maternal age at delivery and KBIT IQ score, presence of two parents in the household, and Hollingshead socioeconomic score.

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