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A Prospective Study of the Effects of Breastfeeding and FADS2 Polymorphisms on Cognition and Hyperactivity/Attention Problems

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Breastfeeding has been associated with improved cognitive functioning. There is a beneficial effect on IQ, and possibly on associated phenotypes such as attention problems. It has been suggested that the effect on IQ is moderated by polymorphisms in the FADS2 gene, which is involved in fatty acid metabolism. In this study we tested the relation between breastfeeding and FADS2 polymorphisms on the one hand and IQ, educational attainment, overactivity, and attention problems on the other hand. IQ at age 5, 7, 10, 12, and/or 18 (n = 1,313), educational attainment at age 12 (n = 1,857), overactive behavior at age 3 (n = 2,560), and attention problems assessed at age 7, 10, and 12 years (n = 2,479, n = 2,423, n = 2,226) were predicted by breastfeeding and two SNPs in FADS2 (rs174575 and rs1535). Analyses were performed using structural equation modeling. After correction for maternal education, a main effect of breastfeeding was found for educational attainment at age 12 and overactive behavior at age 3. For IQ, the effect of breastfeeding across age was marginally significant ($P = 0.05$) and amounted to 1.6 points after correcting for maternal education. Neither a main effect of the FADS2 polymorphisms nor an interaction with

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breastfeeding was detected for any of the phenotypes. This developmentally informed study confirms that breastfeeding is associated with higher educational attainment at age 12, less overactive behavior at age 3 and a trend toward higher IQ after correction for maternal education. In general, the benefits of breastfeeding were small and did not interact with SNPs in FADS2.

Key words: breastfeeding; FADS2; cognition; attention problems; hyperactivity

INTRODUCTION

The positive effects of breastfeeding for the newborn have been shown in many studies and have led the WHO to promote breastfeeding worldwide [WHO, 2003]. A positive association between breastfeeding and cognition has been reported in a substantial number of studies, as reviewed by Horta et al. [2007]. However, studies examining the effect of breastfeeding on IQ are complicated by several confounding effects, most importantly maternal IQ, socioeconomic status (SES) and maternal education [Jain et al., 2002; Der et al., 2006]. Although a meta-analysis and a large randomized trial reported an effect of breastfeeding on cognition independent of several confounders, other studies, including a meta-analysis and a sibling pairs analysis, did not find an association after control for confounding effects [Jain et al., 2002; Der et al., 2006; Horta et al., 2007; Kramer et al., 2008].

During childhood, IQ consistently shows a negative association with Attention Deficit/Hyperactivity Disorder (ADHD) and attention problems (AP) [Polderman et al., 2006]. The effect of breastfeeding on AP and ADHD is less well studied than the effect of breastfeeding on IQ. In a case–control study in 100 children aged 4–11, ADHD cases were breastfed for a significantly shorter period than controls [Kadziela-Olech and Piotrowska-Jastrzebska, 2005]. Furthermore, a prospective cohort study in 500 children found that long-term breastfeeding was associated with fewer ADHD symptoms and improved executive functioning after correction for sociodemographic characteristics of the parents [Julvez et al., 2007]. A study of 1,287 boys aged 6–13 found that inattention was significantly higher among bottle-fed boys, but this effect was not observed for hyperactivity/impulsivity or combined ADHD [Al Hamed et al., 2008]. Finally, a large study (n = 12,167) focusing on the relationship between eczema and ADHD, reported no effect of breastfeeding on ADHD after control for a range of confounding factors including SES [Romanos et al., 2010]. Thus, studies investigating the protective effect of breastfeeding on AP/ADHD have shown inconclusive results. However, the effect of breastfeeding on ADHD is of particular interest in the light of a randomized controlled trial that suggested a hypersensitivity reaction to food as a causal factor for ADHD [Pelsser et al., 2011]. As breastfeeding has been suggested to protect against hypersensitivity reactions, breastfeeding might play a role in the development of AP/ADHD.

The positive effects of breastfeeding on brain development and IQ are thought to be mediated by the presence of long-chain polyunsaturated fatty acids (LC-PUFA) in breast milk, since LC-PUFA's such as docosahexanoic acid (DHA) and arachadonic acid (AA) play a role in neural function. Several studies have shown benefits of supplementation with LC-PUFA's or oily fish intake during pregnancy for ADHD/AP and IQ [Hibbeln et al., 2007; Gale et al., 2008]. Although a meta-analysis on the effect of supplementation did not support a beneficial effect of LC-PUFA's on IQ [Qawasmi et al., 2012], another meta-analysis showed significant improvement of symptoms of ADHD [Bloch and Qawasmi, 2011]. DHA and AA are the products of a process in which essential fatty acids like 3-omega and 6-omega fatty acids are desaturated and elongated [Lattka et al., 2010a,b]. The rate limiting step in the production of DHA and AA is mediated by the FADS enzymes. The importance of these fatty acids for neural development led Caspi et al. [2007] to study two SNPs in the FADS2 gene under the hypothesis that these SNPs could moderate the relationship between breastfeeding and IQ. These two SNPs, rs174575 and rs1535, are in linkage disequilibrium (LD) with other SNPs throughout the promoter and intragenic region of the FADS2 gene. A gene–environment interaction was found in two independent cohorts; children carrying one or two C alleles of the rs174575 SNP showed a clear benefit of breastfeeding, whereas children homozygous for the G allele showed similar IQ scores in the breastfed and non-breastfed groups. Importantly, the interaction effect was significant after correction for maternal IQ and social class and it was shown that the interaction effect was not due to the maternal genotype influencing breast milk quality [Caspi et al., 2007].

Thus far, two studies have attempted to replicate this interaction effect. In a prospective cohort study of 6,000 children, the relationship between breastfeeding and IQ scores at age 8 was found to be modified by the two SNPs in the FADS2 gene [Steer et al., 2010]. However, the interaction effect was in the opposite direction of the effect observed by Caspi et al., with the effect of breastfeeding being significantly larger in children homozygous for the G allele.

A second replication study was performed in a cohort of 700 twin families that provided retrospective data on breastfeeding at age 16–18. Neither a main effect of breastfeeding nor an interaction of the FADS2 gene and breastfeeding was found after control for parental socioeconomic status and education [Martin et al., 2011].

With the present study we contribute to the discussion whether breastfeeding is associated with IQ. We extend the IQ phenotype with associated phenotypes, namely educational attainment (EA) and overactive behavior (OA)/AP throughout childhood and investigate the possible role of FADS2 and its interaction with breastfeeding. Data on IQ, educational attainment (EA) and OA/AP were available for 1,739, 10,669, and 30,561 twins and siblings, respectively. Of these, 1,313, 1,857, and 2,849 individuals were successfully genotyped for the FADS2 SNPs.

MATERIALS AND METHODS

Subjects

The twins included in this study were registered as newborns with the Netherlands Twin Register (NTR) [van Beijsterveldt et al., 2013; Willemsen et al., 2013]. Longitudinal data on health and behavior

are collected starting at registration. Questionnaires are sent out to the parents of the twins at registration and at twins' ages 2, 3, 5, 7, 10, and 12 years. Hereafter, their siblings are invited to participate as well, and both twins and siblings rate their own behavior. At age 18, the parents, twins and siblings are invited to participate in the adult register. Subsamples of twins and siblings are invited to participate in projects that use more elaborate measures such as IQ tests, MRI assessment or neuropsychological test to assess cognitive functioning and development.

IQ data were available for 1,739 individuals (birth cohorts 1974–1998), of whom 1,313 were genotyped for the FADS2 SNPs. IQ was measured in twins at ages 5, 7, 10, 12, and/or 18 and in siblings at age 12 and/or 18. Data on EA at age 12 were available for 10,669 twins and siblings (birth cohorts 1981–1999). Of these, 1,857 were genotyped for the FADS2 SNPs. Data for OA/AP at any time point were available for twins only (n = 30,561, birth cohorts 1986–2004); 28,245 twins had OA data at age 3 and 18,296, 12,834, and 9,143 had data on AP at age 7, 10, and 12 years, respectively. Of these, 2,560, 2,479, 2,423, and 2,226 were genotyped for the FADS2 SNPs. Children suffering from a severe handicap that interfered with daily functioning were excluded from the analyses of OA/AP. Ethnic outliers were excluded from the analyses on the main and interaction effect of FADS2. If available, ethnicity was based on genome wide SNP data, otherwise information on the country of birth of the (grand)parents was used.

Breastfeeding

Breastfeeding status was reported in several surveys. For most twins, breastfeeding information was available from a survey administered at age 2 of the twins. Mothers reported the duration of breastfeeding for each child using the following categories: "no," "less than 2 weeks," "2–6 weeks," "6 weeks–3 months," "3–6 months," and "more than 6 months." For all siblings and a small subgroup of twins, breastfeeding information was based on parental reports from three surveys of the adult register (in 1991, 1995, and 2009) when the children were age 5–33. In one of these surveys, two answer categories were used ("yes" and "no"), while in the other questionnaires the same six answer categories were used as in the young twin register. Breastfeeding was re-coded into two categories: never breastfed ("no" and "less than 2 weeks") versus ever breastfed (all other categories); as this dichotomization led to the highest consistency across raters and across time in individuals measured on multiple occasions. When multiple reports were available, maternal reports were preferred over paternal reports; and the rating closest to the moment of breastfeeding was selected. In children with phenotypic data, the frequency of breastfeeding was 45.0% in twins and 71.4% in siblings. Twin pairs were concordant for breastfeeding in 97.8% of the cases and twin-sibling pairs were concordant for breastfeeding in 72.8% of the cases. Of the children that were breastfed and had detailed information on the duration of breastfeeding, 40.6% were breastfed for more than 3 months (40.1% for twins and 63.5% for siblings). The frequency of breastfeeding was similar in the groups of genotyped and ungenotyped children (43.5% and 45.6%).

Maternal Education

Maternal education was assessed in surveys sent out at twins' ages 3, 7 and 10, on a 13-point scale ranging from primary to post-doctoral education. The most recent measure of maternal education was recoded into one of three categories: low, middle or high educational level. For a small subgroup, data on maternal education were based on surveys from the adult register and recoded into the same three categories as the data from the young register.

Zygosity

For twin pairs in which both twins were genotyped for the FADS2 gene, zygosity was based on a series of SNP markers and repeat polymorphisms or, where available, on genome-wide SNP data [van Beijsterveldt et al., 2013]. In the twin pairs that had no FADS2 data, zygosity was determined by analysis of blood group or DNA polymorphisms in respectively 77.4%, 10.1%, and 6.3% of same-sex twin pairs with data on IQ, EA, and OA/AP. In the remaining cases, zygosity was based on opposite-sex information or a set of questions that gives a correct determination of zygosity in 93% of same-sex twin pairs [Rietveld et al., 2000].

IQ

IQ was measured at ages 5, 7, 10, 12, and/or 18 as part of several studies [Rijsdijk et al., 2002; Polderman et al., 2006; Hoekstra et al., 2007; van Soelen et al., 2011]. At ages 5, 7, and 10, children completed the Revised Amsterdamse Kinder Intelligentie Test (RAKIT) [Bleichrodt et al., 1984]. The short version of the RAKIT was used, containing six subtests with age-appropriate items measuring verbal and nonverbal abilities. At age 10 and 12 the Dutch version of the Wechsler Intelligence Scale for Children-Revised (WISC-R and WISC-R-III) was used [Van Haasen et al., 1986; Wechsler et al., 2002]. Both the complete test, consisting of 6 verbal and 6 nonverbal subtests and a short version, consisting of 6 subtests, were used. At age 18, IQ was assessed using the Dutch version of the Wechsler Adult Intelligence Scale (WAIS-III) [Wechsler, 1997] and the Raven Advanced Progressive Matrices [Raven et al., 1998]. All tests were standardized with equal norms across sex groups. Norms were based on a population sample of same-aged subjects in the Netherlands, except for the Raven scores for which age-corrected standardized scores were calculated based on the NTR dataset. Standardized scores were then calculated for each project separately. For convenience, these *z*-scores were transformed to scores with mean 100 and standard deviation 15.

EA

The CITO-elementary test is a standardized test of Educational Attainment that is administered to 85% of Dutch children in their last year of primary education [CITO, 2002]. The test is taken on 3 consecutive days and covers four domains: Language, Mathematics, Information Processing and World Orientation. The total score ranges between 501 and 550. Bartels et al. [2002] showed that scores on the CITO-elementary test correlate 0.41, 0.50, 0.60, and 0.63 with IQ at ages 5, 7, 10, and 12, respectively.

OA and AP

An age-appropriate version of the Child and Behavior Checklist (CBCL) was included in the questionnaires that were sent out to mothers of twins at ages 3, 7, 10, and 12 of the children. The Overactive scale at age 3 (OA) contains 5 items describing overactive and inattentive behaviors and the Attention Problems scale (AP) at ages 7, 10, and 12 contains 11 items that describe hyperactive and inattentive behaviors [Achenbach, 1991, 1992; Verhulst et al., 1996; Koot et al., 1997]. The sum score of the AP scale has been shown to converge with a DSM-based ADHD diagnosis [Derks et al., 2006]. As the two scales were analyzed simultaneously, scores were standardized by subtracting the mean score and dividing the outcome by the standard deviation.

FADS2 Genotyping

The twins and siblings included in the study were genotyped as part of several projects. Most genotype data come from the SNP fingerprint chip that was used to determine zygosity and identify sample switches using a set of SNPs in candidate genes [see van Beijsterveldt et al., 2013]. The remaining FADS2 genotype data come from genome-wide SNP arrays that were imputed against the 1,000 genomes references set (June 2011, all panels) after stringent quality control. SNP quality control before imputation included filtering on the following criteria: HWE P-value > 0.00001; MAF > 0.01; SNP call rate > 0.95; SNP concordance rate $< 2\%$; Mendel error rate $< 2\%$ and allele frequency difference with reference set < 0.20. C/G and A/T SNPs were only included if MAF < 0.35. Samples were included if the missing rate was $< 10\%$, known gender was in line with the X-chromosome genotypes, IBS/IBD relationships were in line with known family relations and there were no issues of excessive heterozygosity or IBS sharing. The rs174575 and rs1535 SNPs were imputed with high quality (R^2 for rs174575 $= 0.93$, R^2 for rs1535 $= 0.98$). Genotypes of the rs174575 and rs1535 SNPs were in Hardy–Weinberg equilibrium ($P > 0.05$) and allele frequencies were comparable to HapMap.

Analyses

To assess the effect of breastfeeding on IQ, EA, and OA/AP, different structural equation models were fit; here, the EA, IQ and OA/AP scores were regressed on maternal education, sex and breastfeeding. To correct for the dependency in the data, covariances across ages and family members were estimated. For the analysis of EA, a single observation at age 12 was available for both twins and siblings, and a 3×3 matrix was estimated. In this matrix, the variance of EA and the covariances of EA within twin-sibling and twin pairs were estimated, with separate estimates for monozygotic and dizygotic twin pairs. For the analyses of IQ and OA/AP, longitudinal data were available, and we fit a model in which (co)variances were estimated across all available age groups. Thus, in the IQ analyses for instance, a 12×12 full covariance matrix was estimated (5 ages $\times$ 2 twins + 2 ages $\times$ 1 sibling). Separate covariance matrices were estimated for twin-sibling, monozygotic twin and dizygotic twin pairs, consisting of covariances between family members. The covariance across ages within individuals was constrained to be equal across twins and siblings.

The effects of breastfeeding, sex and maternal education were estimated as fixed effects in the total sample of genotyped and ungenotyped individuals. For IQ and OA/AP, it was first tested whether the effect of breastfeeding could be constrained to be equal across ages without a significant deterioration of model fit. If this was allowed it was tested whether the parameter could be excluded from the model. To assess the effect of FADS2 and the FADS2-by-breastfeeding interaction, similar models were fitted to the data of the subgroup with available rs1535 and rs174575 genotypes, with the SNP and SNP-by-breastfeeding effect as fixed effects in the model (see Fig. 1). For IQ, it was first tested whether the breastfeeding, SNP and SNP-by-breastfeeding effect could be constrained to be equal across ages without a significant deterioration of model fit, both for each effect separately and for the breastfeeding, SNP and SNP-by-breastfeeding effects together. If this was allowed, it was tested whether excluding a predictor from the model across all ages led to a significantly worse model fit. For OA/AP, the effect of breastfeeding was significantly different across age and the breastfeeding and SNP-by-breastfeeding effect was therefore tested for each age separately. Predictors whose exclusion did not lead to a significant decline in model fit where left out from subsequent models. The two SNPs were tested separately in an additive and a recessive model. The interaction of breastfeeding and FADS2

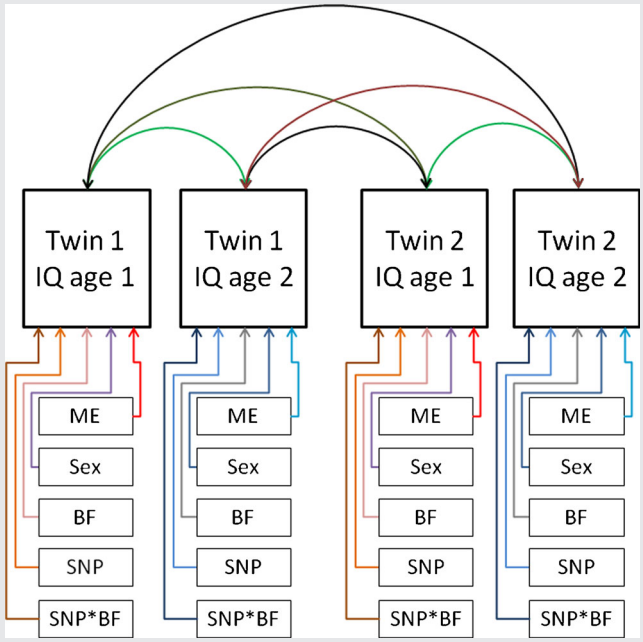

FIG. 1. Model to estimate the effect of maternal education (ME), sex, breastfeeding (BF), SNP, and SNP-by breastfeeding (SNP*BF) on IQ. For the sake of clarity only two time points and two family members are shown. Included but not shown in the model are the within person variance of IQ and the intercept at each time point. Paths that have the same color in the figure are constrained to be equal in the full model. Covariances within twin pairs were estimated for monozygotic and dizygotic twins separately.

genotype was tested in the presence of main effects of breastfeeding and FADS2.

The Mx software package was used for all analyses [Neale et al., 2006]. Mx uses a full information maximum likelihood (FIML) method to fit a specified model to the data. The difference in minus two times the log-likelihood (−2LL) between two models has an asymptotic χ^2 distribution with the degrees of freedom (df) equaling the difference in parameters between the two models. The fit of nested models can therefore be compared using the log-likelihood ratio test (LRT). As we analyzed three phenotypes, α-level of 0.05 was divided by the number of phenotypes: 0.05/3 = 0.017.

We performed a simulation study to estimate the power to detect the interaction effect described by Caspi et al. in the current dataset. The effect sizes for breastfeeding, rs174575 and its interaction were calculated based on the descriptive statistics reported by Caspi et al.; the interaction effect explained 0.3% of the variance in IQ in their dataset. We simulated 10,000 datasets that mimicked the structure of the current dataset with regard to sample size, family relations, longitudinal data structure and patterns of missingness, using the statistical package R (script available in Supplementary Material I). Effects were assumed to be equal across age and were not corrected for sex and maternal education, as only unadjusted effect sizes could be derived from the study by Caspi et al.

RESULTS

The effect of breastfeeding on IQ was not significantly different across the different ages. After correction for sex and maternal education, the effect of breastfeeding was significant for EA at age 12 and marginally significant for IQ across all ages ($P = 0.05$), see Table I. The effect of breastfeeding on OA/AP differed significantly across age and reached significance for OA at age 3. After correction for sex and maternal education, breastfed children scored on average 1.6 points (95% CI: −0.00 to 3.14) higher on IQ, 1.3 points (95% CI: 0.93–1.69) higher on the EA scale and 0.15 points (95% CI: 0.09–0.21) lower on the OA scale.

For the children with genotype data, the mean IQ, EA, and OA/AP scores for breastfed and non-breastfed children are shown in Table II, for each genotype group of rs174575 separately. This SNP showed the most convincing results for a gene–environment interaction in previous studies. In Figure 2 the same results are shown graphically for one measurement of each phenotype, OA at age 3 was selected because this was the only age for which a significant breastfeeding effect was found in the OA/AP analyses and IQ at age 18 was selected as this was the age at which most IQ data were available. Following previous studies, we first tested a recessive model. The main and interaction effects of breastfeeding and the child's genotype on IQ did not significantly differ across age, either when tested separately or with the three effects together. The main effect of the SNP and the SNP-by-breastfeeding interaction were not significant for IQ, EA, and OA/AP for rs174575 and rs1535 (Table III). The point estimate of the interaction effect in the IQ analyses (not breastfed versus breastfed children coded 0–1 and CC/CG versus GG genotypes coded 0–1), was −5.30 (95% CI: −11.32 to 0.73). The additive models also showed no significant results (Supplementary Table SI). Results for the SNP and SNP-by-breastfeeding effect did not change meaningfully when not corrected for maternal education and sex (results available upon request).

The simulation study indicated that, at a significance level of 0.05, we were at 53% power to detect the previously reported interaction effect on IQ. For EA and OA/AP, we had 54% and 86% power to detect an interaction effect similar in effects size as the interaction effect on IQ reported by Caspi et al.

TABLE I. Model Fitting Results for the Effect of Breastfeeding in the Total Group With Available Data, Adjusted for Maternal Education and Sex

Model	Versus model	−2LL	df	χ^2	Δdf	*P*-value	Conclusion
IQ							
1. Full model		28605.199	3,681				
2. Equal β's BF across age	1	28605.963	3,685	0.764	4	0.943	Effect of BF is the same across age
3. Drop β breastfeeding	2	28609.804	3,686	3.841	1	0.050	Effect of BF is marginally significant
EA							
1. Full model		72965.872	10,661				
2. Drop β breastfeeding	1	73010.491	10,662	44.619	1	<0.001	Effect of BF is significant
OA/AP							
1. Full model		165167.102	68,472				
2. Equal β's BF across age	1	165179.228	68,475	12.126	3	0.007	Effect of BF is different at different ages
3. Drop β BF age 3	1	165193.105	68,473	26.003	1	<0.001	Effect of BF is significant at age 3
4. Drop β BF age 7	1	165167.368	68,473	0.266	1	0.606	Effect of BF is not significant at age 7
5. Drop β BF age 10	4	165168.165	68,474	0.797	1	0.372	Effect of BF is not significant at age 10
6. Drop β BF age 12	5	165168.705	68,475	0.540	1	0.462	Effect of BF is not significant at age 12

EA, educational attainment; OA, overactive behavior; AP, attention problems; BF, breastfeeding.

TABLE II. Mean and Standard Deviation of IQ, Educational Attainment (EA), Overactive Behavior (OA), and Attention Problems (AP) Scores for Breastfed and Non-Breastfeed Children and Genotype Groups of rs174575 Separately

		rs174575 CC carriers				rs174575 CG carriers				rs174575 GG carriers			
		Not breastfed		Breastfed		Not breastfed		Breastfed		Not breastfed		Breastfed	
		n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
IQ	Age 5	205	99.5 (14.9)	131	101.0 (13.8)	143	100.5 (14.9)	105	103.6 (14.4)	25	104.4 (10.7)	12	107.1 (10.7)
	Age 7	77	100.3 (14.3)	69	102.1 (14.5)	62	99.7 (14.2)	38	103.3 (15.1)	9	107.5 (12.3)	5	103.8 (8.9)
	Age 10	139	98.9 (13.6)	120	98.6 (15.4)	86	97.2 (14.3)	74	102.7 (13.5)	13	103.2 (13.2)	14	108.6 (10.7)
	Age 12	238	98.5 (14.0)	182	99.6 (14.5)	144	98.9 (13.9)	128	104.3 (14.4)	28	101.4 (13.1)	17	104.0 (12.9)
	Age 18	278	98.5 (15.5)	169	102.6 (12.9)	179	100.0 (15.2)	152	103.4 (13.6)	40	104.6 (13.4)	21	97.1 (13.9)
EA	Age 12	539	537.0 (8.2)	449	539.1 (8.4)	405	536.9 (8.8)	346	539.9 (8.0)	59	536.0 (9.3)	54	539.6 (8.1)
OA	Age 3	813	2.74 (2.18)	602	2.40 (2.14)	528	2.95 (2.22)	456	2.35 (2.10)	78	2.91 (2.53)	76	2.13 (1.81)
AP	Age 7	779	2.99 (3.00)	579	2.66 (2.89)	525	3.08 (2.99)	438	2.70 (3.10)	79	2.99 (3.28)	74	2.01 (2.03)
AP	Age 10	764	3.03 (3.08)	569	2.46 (2.87)	521	3.23 (3.34)	419	2.78 (3.37)	77	3.34 (3.28)	68	2.29 (2.44)
AP	Age 12	698	2.63 (2.77)	524	2.24 (2.80)	467	2.94 (3.14)	397	2.34 (2.71)	72	2.54 (3.30)	63	2.10 (1.73)

EA, educational attainment; OA, overactive behavior; AP, attention problems.

DISCUSSION

Breastfeeding was associated with educational attainment at age 12 and overactive behavior at age 3. For IQ at age 5–18, the effect was marginally significant after correction for maternal education. Polymorphisms in the FADS2 gene did not moderate the relationship between breastfeeding and IQ, educational attainment or overactive behavior. In addition, no main effects of the SNPs were found.

The positive effect of breastfeeding on IQ and EA is in line with findings of previous studies, including a study on the effect of breastfeeding on EA from our own group [Horta et al., 2007; Kramer et al., 2008; Bartels et al., 2009]. The effect of breastfeeding did not significantly differ across age, in line with a meta-analysis that found no moderation of the effect of breastfeeding by age at measurement [Anderson et al., 1999]. A small protective effect of breastfeeding after correction for maternal education was found for OA at age 3, but not for AP at age 7, 10, and 12. Thus far, several studies on smaller samples suggested an effect of breastfeeding on ADHD/AP, but a large cohort study did not detect such an effect if the results were corrected for a range of confounding factors including SES [Kadziela-Olech and Piotrowska-Jastrzebska, 2005; Julvez et al., 2007; Al Hamed et al., 2008; Romanos et al., 2010]. The fact that we found a significant effect of breastfeeding only on OA at age 3 could suggest that the effect of breastfeeding is only present at a young age, or that the effect is specific to the OA scale. There are only 2 overlapping items between the OA and the AP scale; however, both scales describe hyperactive and inattentive behaviors with no clear difference in the overall content of the scales.

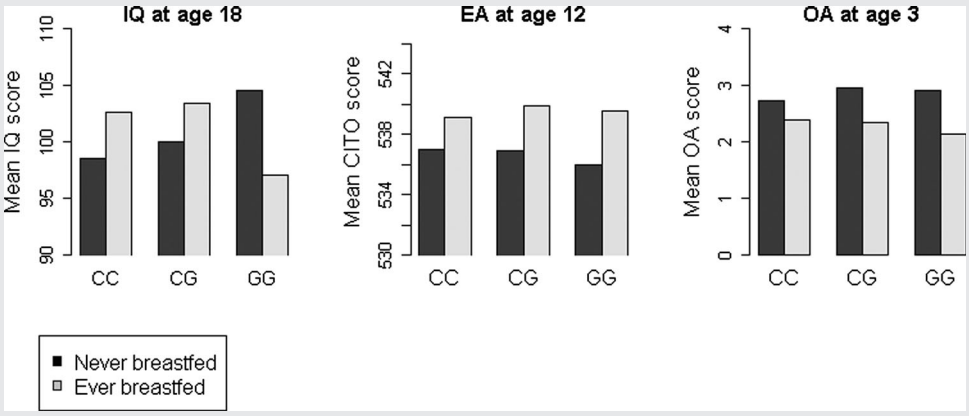

FIG. 2. Mean IQ (age 18), educational attainment (EA, age 12) and overactive behavior (OA, age 3) scores, for breastfed and non-breastfed children and genotype groups of rs174575 separately.

TABLE III. Model Fitting Results for the Recessive Model of rs174575 and rs1535, Adjusted for Maternal Education and Sex

		rs174575					rs1535				
Model	Versus model	−2LL	df	χ^2	Δdf	*P*-value	−2LL	df	χ^2	Δdf	*P*-value
IQ											
1. Full model		22070.409	2,818				22172.311	2,831			
2. Equal β's across age	1	22079.993	2,830	9.584	12	0.652	22185.362	2,843	13.051	12	0.365
3. Drop β SNP*BF	2	22082.960	2,831	2.967	1	0.085	22188.678	2,844	3.316	1	0.069
4. Drop β SNP	3	22083.582	2,832	0.622	1	0.430	22189.577	2,845	0.899	1	0.343
EA											
1. Full model		12547.612	1,842				12569.755	1,844			
2. Drop β SNP*BF	1	12547.821	1,843	0.209	1	0.648	12569.921	1,845	0.166	1	0.684
3. Drop β SNP	2	12548.077	1,844	0.256	1	0.613	12572.235	1,846	2.314	1	0.128
OA/AP											
1. Full model		22092.026	9,612				22134.252	9,628			
2. Drop β SNP*BF age 3	1	22092.974	9,613	0.948	1	0.330	22134.603	9,629	0.351	1	0.554
3. Drop β SNP*BF age 7	2	22094.743	9,614	1.769	1	0.184	22135.309	9,630	0.706	1	0.401
4. Drop β SNP*BF age 10	3	22094.774	9,615	0.031	1	0.860	22135.969	9,631	0.660	1	0.417
5. Drop β SNP*BF age 12	4	22095.263	9,616	0.489	1	0.484	22139.407	9,632	3.438	1	0.064
6. Equal β SNP across age	5	22097.483	9,619	2.220	3	0.528	22147.392	9,635	7.985	3	0.046
7. Drop β SNP	6	22097.893	9,620	0.410	1	0.522	22147.393	9,636	0.001	1	0.975

EA, educational attainment; OA, overactive behavior; AP, attention problems; BF, breastfeeding; SNP, single nucleotide polymorphism.

The results on the main effects of FADS2 on OA/AP are in line with a meta-analysis of genome-wide association studies on ADHD that showed no evidence for an association with any of the FADS2 SNPs included in the study [Neale et al., 2010]. Significant associations between FADS2 and AP/ADHD have only been reported for rs498793, which is in low LD with rs1535 and rs174575 [Brookes et al., 2006].

The current study did not replicate the interaction effect between breastfeeding and the FADS2 genotype found by Caspi et al. [2007]. A previous replication effort [Steer et al., 2010] reported an interaction effect in the opposite direction, and another replication effort [Martin et al., 2011] detected no interaction effect between FADS2 and breastfeeding on IQ. Martin et al. [2011] stressed that the plausibility of the interaction effect of FADS2 and breastfeeding is not extensively supported given the fact that all studies to date have failed to detect a main effect of the FADS2 SNPs on IQ. Munafo et al. [2009] showed that GxE interaction is unlikely to be present in the absence of a main effect of the genetic factor, given sufficient power. That is, when the environmental factor is not rare, the effect of the genotype in the exposed group will have a diminished but detectable effect in the total group of exposed and unexposed individuals and will thus be reflected as a significant main effect in the total group. However, it should be noted that the argument by Munafo et al. specifically focused on the case in which one of the groups (i.e., the unexposed group) shows no differences in the outcome variable across genotype groups. This is not the case for the results reported by Caspi et al.; breastfed children showed the lowest IQ scores in the GG group, whereas non-breastfed children showed the highest IQ scores in the GG group. This leads to the absence of a main effect of the genotype in the presence of a true interaction effect, as the genotype is advantageous in the non-breastfed group and non-advantageous in the breastfed group.

The sample size of the current study was smaller than the sample size of some of the previous studies. The simulation study showed that, at a significance level of 0.05, we were at 53%, 54%, and 86% power to detect an interaction effect on IQ, EA and OA/AP, respectively, assuming an interaction effect on IQ similar in size to the effect reported by Caspi et al. Altogether, the lack of an interaction effect in the present study and in the study by Martin et al. add to the conflicting results of the earlier studies [Caspi et al., 2007; Steer et al., 2010]. The observed pattern is in line with the so called "winner's curse" phenomenon. In a context of low power in which claims of discovery are based on thresholds of statistical significance, newly discovered associations are prone to overestimate true effect sizes [Ioannidis, 2008]. Further replication efforts and a successive meta-analysis are needed to further evaluate the possible interaction effect of FADS2 and breastfeeding on IQ.

Limitations

Participants included in the OA/AP analysis were born between 1986 and 2004. Modern formula contains DHA and AA since the early 2000s, which could lead to a smaller effect of breastfeeding on OA/AP in the later cohorts. While maternal education is a reasonable proxy for maternal IQ, the latter is to be preferred according to some researchers [Der et al., 2006]. Although this could be regarded as a limitation for the analysis of the effect of breastfeeding, for the replication effort of the FADS2-by-breastfeeding effect this is of little importance, as the results by Caspi et al. were reported to be significant without correction for maternal IQ.

In conclusion, a protective effect of breastfeeding was found for overactive behavior at age 3 and educational attainment at age 12. These results were obtained after correction for maternal education. For IQ at ages 5, 7, 10, 12, and 18, the effect was marginally significant after correction for maternal education. No main effect of SNPs in FADS2 nor an interaction between FADS2 SNPs and breastfeeding was detected for any of the phenotypes.

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