Cognitive Function in Adolescence: Testing for Interactions Between Breast-Feeding and *FADS2* Polymorphisms

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Objectives: Breast-fed C-allele carriers of the rs174575 single nucleotide polymorphism in the fatty acyl desaturase 2 (*FADS2*) gene have been reported to show a 6.4 to 7 IQ point advantage over formula-fed C-allele carriers, with no effect of breast-feeding in GG carriers. An Australian sample was examined to determine if an interaction between breast-feeding and the rs174575 single nucleotide polymorphism had any effect on IQ. **Method:** This hypothesis was tested in more than 700 families of adolescent twins assessed for IQ and breast-feeding, birth weight, and *FADS2* polymorphisms, and parental socioeconomic status and education, and maternal *FADS2* status. **Results:** No significant evidence for a moderating effect on IQ of rs174575 C-carrier status and breast-feeding was found, and there no effects of maternal *FADS2* status on offspring IQ. In addition, no main effects of any *FADS2* polymorphisms on IQ were found when the genotype was kept as two-homozygote and one-heterozygote categories and indeed no evidence for effects of breast-feeding on IQ scores after controlling for parental socioeconomic status and education. The investigation was extended to two additional *FADS2* polymorphisms (rs1535 and rs174583), but again, although these polymorphisms code alleles affecting fatty acid metabolism, no main or interaction effects were found on IQ. **Conclusion:** These results support the view that apparent effects of breast-feeding on IQ reflect differential likelihood of breast-feeding as a function of parental education and did not support the predicted interaction effect of *FADS2* and breast-feeding on IQ. J. Am. Acad. Child Adolesc. Psychiatry, 2011; 50(1):55–62. **Key Words:** Breast-feeding, IQ gene-environment interactions, fatty acid metabolism, *FADS2*

A core topic in the fields of pediatric medicine, developmental psychology, and cognitive science is the potential benefit of breast-feeding. Initial support for a beneficial effect of breast-feeding on IQ has been provided by observational studies showing evidence of higher IQ in children who were breast-fed.[1-7] This apparent effect of breast-feeding on cognitive development has been supported by biological studies indicating that breast milk components including long-chain polyunsaturated fatty acids (LC-PUFAs) may influence early brain development.[8] Breast-feeding, however, is confounded by background factors such as socioeconomic status (SES) and parental education, which are associated with IQ and the decision to breast-feed, and a series of studies has suggested that these confounders account for all or most of the association of breast-feeding with cognitive ability.[9-15] Critically, Der et al.[9] conducted a meta-analysis of previous studies finding that maternal IQ alone accounted for 72% of the apparent effects of breast-feeding on IQ. This group also presented data indicating that when maternal IQ was taken into account, there was no longer any significant association of breast-feeding with IQ.

Recently, Caspi et al.[16] suggested a moderating effect of genetics on breast-feeding effects, such that the effect of breast-feeding would be contingent on the allelic status of genes involved in fatty acid metabolism, with some children showing an effect of breast-feeding, whereas oth-

Supplemental material cited in this article is available online.

ers, with different fatty acid metabolisms, would show no effect of milk versus formula feeding. They investigated two single nucleotide polymorphisms (SNPs), rs174575 and rs1535, which are in strong linkage disequilibrium with SNPs in the promotor and 5′ regions of the fatty acyl desaturase 2 gene (*FADS2*) and the very similar adjacent gene *FADS1*. Their key finding was an IQ advantage of 6.4 to 7 IQ points for breast-fed versus non-breast-fed infants among children carrying one or more C allele of the rs174575 SNP, but with no main effect of carrier status and no effect of breast-feeding among GG homozygotes. Since Caspi et al.[16] reported this finding, one attempt to replicate has been reported. In a study of 5,934 children 8 years old, Steer et al.[17] found no main effect of rs174575 on childhood IQ but did find a significant interaction such that breast-fed children homozygous for the G allele of rs174575 performed better than formula-fed GG-homozygous children by 5.8 IQ points. This study, then, failed to replicate the sensitivity of C-allele carriers to breast-feeding.

FADS2 is one of three members of the *FADS* gene cluster[18] that encode for rate-limiting enzymes in the synthesis of ω-3 LC-PUFAs[18-23] that are involved in a wide range of cellular processes including docosanoid and eicosanoid synthesis and gene expression regulation.[21,24-26] An accumulation of these LC-PUFAs takes place in the brain during the first months after birth,[27] and this is positively associated with better development of neural function.[25,27-32] It has also been shown that children who are breast-fed have a higher concentration of LC-PUFAs than children fed unsupplemented formula.[33,34] Breast-feeding may also affect IQ through maternal effects on LC-PUFA metabolism, which influence the fatty acid composition of breast milk. Xie and Innis[23] reported an association of polymorphisms in the *FADS* gene cluster (including rs174583 tested in the present study) with altered ω-6 and ω-3 essential fatty acid components in breast milk.

In the present study, we examined the effect of breast-feeding and confounding factors on IQ in our Australian twin sample (N = 1431), testing for a predicted gene-by-environment interaction of *FADS2* polymorphisms rs174575, 174583, and rs1535 on cognitive development in breast-fed and nonbreast-fed children. In addition, we extended this investigation to examine main effects and effects of duration of breast-feeding on offspring IQ and testing for effects of maternal *FADS2* genotypes on IQ variation in offspring.

METHOD

Participants

Twins and their siblings were initially recruited as part of ongoing population studies of melanoma risk factors and cognition in the greater Brisbane area.[35] Data examined in the present study were collected before July 2008 as part of the cognition study (since 1996; e.g., Luciano et al.[36]). This sample has previously been shown to have adequate power for detecting gene effects on cognition in linkage[37] and association[38] studies. At the time the study was performed 1,838 individuals had been assessed for full-scale IQ (FSIQ). Breast-feeding data were available for 1,431 of the 1,838 participants (678 male and 753 female) comprising 720 twin pairs (278 monozygotic pairs and 442 dizygotic pairs) ranging in age from 15 to 22 years (mean ± standard deviation [SD] = 16.28 ± 0.45 years). A range of other measurements (including SES, paternal education, maternal education, gestational age, and birth weight) were available for more than 99% of the sample and 76% of these individuals have been genome-scanned on the Illumina 610-QUAD SNP (Illumina Inc., San Diego, CA) array (monozygotic and dizygotic twin pairs due to a current project examining copy number variations in monozygotic twins). No major differences in FSIQ, breast-feeding, and confounders were found between genotyped and nongenotyped individuals (Table S1, available online).

Zygosity for twin pairs of the same sex was initially determined by genotyping nine polymorphic microsatellite markers (AmpF1STR Profiler Plus Amplification kit; Applied Biosystems, Foster City, CA) and subsequently confirmed by genomewide association scan of most pairs. Exclusion criteria for the cognition study included a significant head injury, neurological or psychiatric conditions, history of substance abuse/dependence, and/or taking medications with significant central nervous system effects. This information was obtained through parental report. Informed written consent for all measurements was obtained from participants and their parents/guardian if participants were younger than 18 years. Ethics approval for these studies was obtained from the human research ethics committee of Queensland Institute of Medical Research.

Measurements

IQ. The FSIQ was assessed using the Multi-dimensional Aptitude Battery (MAB).[39] The MAB is a general intelligence test designed to mirror the Wechsler Adult Intelligence Scale–Revised[40] and presented in a multiple-choice format. Participants completed three verbal (information, arithmetic, vocabulary) and two

(spatial, object assembly) performance subtests, which were combined in the present study to form a full-scale estimate of ability. Twins took the MAB as close as possible to their 16th birthday when they came to Queensland Institute of Medical Research for the cognition study. The average FSIQ of the entire sample assessed (N = 1,838) was 112 (SD = 12.8). Similarly, the average FSIQ of the subsample used in the present study (n = 1431) was 112 (SD = 13.3). FSIQ was normally distributed for both samples. The observed higher average is likely due to the fact that the MAB test was created and normalized for Canadian samples and therefore results on this test may differ when used in a different country. In addition, the presence of an ascertainment bias cannot be excluded. However, the higher IQ mean does not affect the representativeness of this sample because IQ follows a normal distribution and scores range from 77 to 153. Further details of the IQ testing procedure have been previously published.[41,42]

Breast-feeding. Breast-feeding was assessed by a questionnaire completed by the mother when the twins were 12 or 16 years old as part of the melanoma or the cognition study, respectively. In the case of twins for whom this information was not available and who had previously completed the cognition study (66% of total breast-feeding sample, n = 2,026), the questionnaire was mailed to the mother. Feeding practice was reported independently for each twin and was based on four categories: exclusively formula-fed, any breast-feeding between birth and 3 months, exclusively breast-fed for 3 to 6 months, and exclusively breast-fed for 6 months or longer.

Demographic and Gestational Data. In addition to breast-feeding, birth weight of the twins and gestational age (number of weeks) was ascertained by the mothers when the twins came in for testing at 12 or 16 years old. Parental SES was scaled (0 to 100; 100 being the top of the scale) using the Australian Standard Classification of Occupation first edition (1986; 37% of sample) and second edition (1997) provided by the Australian Bureau of Statistics (http://www.abs.gov.au/). The highest parental SES score was selected as the familial SES score for this analysis. Familial SES scaled scores from the first and second editions were standardized into *Z*-scores independently and the *Z*-scores were merged together to obtain an SES score for the full sample. Parental education was scored on a scale from 1 to 8, with a score of 1 representing 7 or fewer years of schooling and a score of 8 representing university postgraduate training. Maternal education was chosen as a substitute for maternal IQ (which was not available) because it has been shown to correlate substantially with IQ (around 0.8) in a large sample of 70,000 individuals.[43] However, the interaction results presented by Caspi et al.[16] are reported without controlling for maternal IQ. In their report they mentioned only that maternal IQ had no significant effect on their children's IQ because the interaction between *FADS2* and breast-feeding remained significant. Thus our controlling for an IQ proxy (maternal education) is entirely incidental in the replication.

Genotyping

Genotyping was performed using the Illumina 610-QUAD SNPs chip as part of ongoing genomewide association scan projects. Genotypes of *FADS2* polymorphisms rs174575 (imputed with an R^2 imputation quality score of 1), rs1535 (on chip), and rs174583 (imputed with an R^2 imputation quality score of 0.98) were then extracted from our genomewide association scan data for the purposes of this study. Rigorous quality controls were applied to the data before and after imputation in MACH 1.0 (http://www.sph.umich.edu/csg/abecasis/MACH/) and SNPs were removed if the following criteria applied: a minor allele frequency <0.01, a significant Hardy-Weinberg violation ($<.000001$), an R^2 imputation quality score <0.3 (R^2 represents the square of correlation between the imputed and measured genotypes). Full details on the quality control and imputation procedure applied for this dataset and have been previously described.[44]

Statistical Analyses

Analyses were performed in Mx[45] to account for twin family structure (zygosity) and covariates. Sex, age, SES, paternal education, maternal education, gestational age, birth weight, and breast-feeding were fitted independently in a series of regression models in which their effects on FSIQ were tested by observing the difference in $-2 \times$ log-likelihood between the full model and a nested model in which each variable was dropped in turn using a χ^2_1 test. The entire sample (including nongenotyped individuals) was used in all analyses to maximize power in modeling of the trait means and variances.

To minimize the need to correct for multiple testing, the possible moderating effect of breast-feeding on IQ by interactions with *FADS2* polymorphisms was tested using Mx separately for each SNP, with significant covariates (sex, SES, maternal education, paternal education, and birth weight) included, in addition to genotype (coded as dominant effects—major allele carrier versus minor allele homozygote, e.g., rs174575 C carriers versus GG homozygotes), breast-feeding, and genotype-by-breast-feeding interaction effects. In subsequent analyses, the main effects of *FADS2* polymorphisms rs174575, rs1535, and rs174583, with FSIQ and FSIQ adjusted for covariates, were examined using MERLIN[46] to account for zygosity status with the fast association comment (—*fastassoc*).

Power Analysis

A power analysis was conducted in G*Power 3.1.[47] We estimated that the power to detect, at the .05 level, the 6.5

TABLE 1 Results Showing Effects of Age, Sex, Socioeconomic Status (SES), Paternal Education, Maternal Education, Gestational Age, Birth Weight, and Breast-Feeding on full-scale IQ (FSIQ), FSIQ Adjusted for Sex, and FSIQ Adjusted for All Variables

	Unadjusted FSIQ				FSIQ Adjusted for Sex				FSIQ Adjusted for All Covariates			
Variables	**n**	**$\Delta\chi^2$**	**Δdf**	***p***	**n**	**$\Delta\chi^2$**	**Δdf**	***p***	**n**	**$\Delta\chi^2$**	**Δdf**	***P***
Sex	1,431	22.9	1	1.75×10^{-6}	—	—	—	—	—	—	—	—
SES	1,426	70.2	1	5.33×10^{-17}	1,426	69.1	1	9.22×10^{-17}	—	—	—	—
Paternal education	1,414	74.0	1	7.69×10^{-18}	1,414	71.0	1	3.53×10^{-17}	—	—	—	—
Maternal education	1,430	67.7	1	1.90×10^{-16}	1,430	63.7	1	1.42×10^{-15}	—	—	—	—
Gestational length	1,431	3.9	1	0.047	1,431	2.8	1	0.096	—	—	—	—
Birth weight	1,417	40.5	1	1.99×10^{-10}	1,417	34.1	1	5.37×10^{-9}	—	—	—	—
Breast-feeding	1,427	9.0	1	2.74×10^{-3}	1,427	8.7	1	3.06×10^{-3}	1,405	2.6	1	.104

Note: Breast-feeding effect on unadjusted FSIQ (β = 1.14, 95% confidence interval = 0.39-1.88), FSIQ adjusted for sex (β = 1.12, 95% confidence interval = 0.37-1.85), and FSIQ adjusted for all covariates (β = 0.58, 95% confidence interval = −0.12 to 1.29). N = number of individuals.

IQ point (SD = 0.43) mean difference previously described by Caspi et al.[16] between breast-fed children who carry an rs174575 C allele (n = 871) and nonbreast-fed children who carry an rs174575 C allele (n = 159) was 100% in our sample. However, this is an overestimate because twins are not independent individuals. If the same power calculation is performed with half the number of individuals in each group, which is extremely conservative and equivalent to considering each twin pair as a single individual, the power was 97%.

RESULTS

A significant effect of sex was found on FSIQ, with male subjects having a higher FSIQ mean than female subjects (mean ± SD = 114.0 ± 12.8 versus 111.1 ± 12.6; Table 1). Other covariates showing significant effects on FSIQ (adjusted for sex) included birth weight, SES, and paternal and maternal education (Table 1). Education effects remained significant when FSIQ (adjusted for sex) was further adjusted for SES in the model ($p = 1.49 \times 10^{-5}$ and $p = 2.30 \times 10^{-5}$ for paternal and maternal, respectively). The effect of gestational age on FSIQ was not significant (Table 1). Similar results were obtained when tests were performed on unadjusted FSIQ scores (Table 1).

Breast-feeding was significantly associated with FSIQ scores, but this effect was no longer significant after controlling for SES, paternal education, maternal education, gestational age, and birth weight (Table 1). No effect of duration of breast-feeding on FSIQ was found (Figure 1).

Turning to the critical predicted interaction effect of rs174575 status with breast-feeding, Mx modeling showed no significant support for the interaction, whether treating FSIQ scores adjusted for sex only or adjusting for all background covariates (Table 2). In addition, when maternal education (proxy for maternal IQ) was removed from the analysis, breast-feeding and rs174775 by feeding method still did not have a significant effect on FSIQ (p = .33 and p = .49, respectively). Similarly, for SNPs rs1535 and rs174583, no interaction effects for breast-feeding were found using sex-adjusted or fully adjusted FSIQ (Table 2). Additional comparison data for children grouped according to breast-feeding and genotype (rs174575, rs1535, and rs174583) for FSIQ score and covariates can be found in Table S2, available online, and results from Table 2 can be found in terms of change of χ^2 and degree of freedom in Table S3, available online.

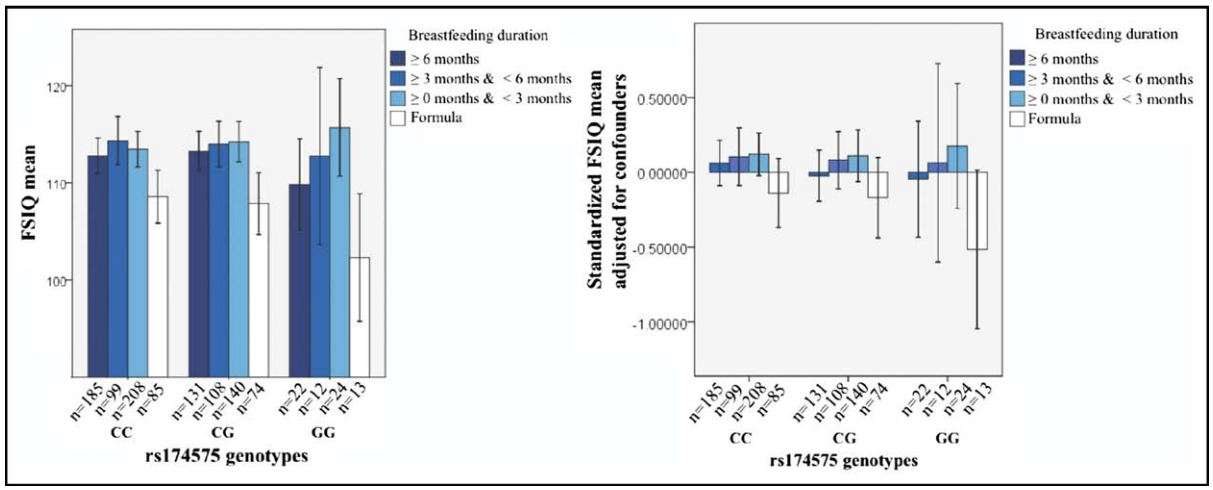
Analyses in MERLIN indicated no significant association of the *FADS2* rs174535 polymorphism with FSIQ scores, whether adjusted for covariates or not (p = .60 to .91; rs1535, p = .51 to .82; rs174535, p = .45 to .78).

To test the hypothesis that *FADS2* might influence offspring IQ through maternal fatty acid metabolism rather than infant metabolism, we tested for an association between maternal variation in each of the three *FADS2* polymorphisms and offspring FSIQ scores, adjusted for sex only. No effects of maternal *FADS2* status were found (p = .34 to .92; Table 3).

DISCUSSION

In the present study we attempted to replicate associations of *FADS2* polymorphisms previ-

FIGURE 1 (Left) No dose-response relation was found between duration of breast-feeding and full scale IQ (FSIQ), as indicated by the overlapping error bars between the different duration groups in the three rs174575 genotype categories. (Right) Once FSIQ was adjusted for confounders (sex, socio-economic status (SES), paternal and maternal education, and birth weight), there was no significant evidence of a FSIQ mean differences between C-allele carriers and GG homozygotes for the breast-fed groups (breast-fed for ≥6 months, ≥3 months, or <3 months) and the formula-fed group. This is indicated by the overlapping error bars within and between the different genotype categories. Note: IQ scores in the left panel are not adjusted for confounders. IQ scores in the right panel are standardized residuals after adjustment for confounders. Error bars represent 95% confidence intervals.



ously reported to benefit early cognitive development in breast-fed children.[16] Similarly to Caspi et al.[16] and Steer et al.,[17] we found no main effect of *FADS2* alleles on IQ under additive or dominant models. We were, however, unable to replicate support for an interaction between *FADS2* polymorphisms and breast-feeding. The lack of interaction status at these polymorphisms with breast-feeding status suggests that no significant gain of IQ occurs for breast-fed children who carry the common allele of these SNPs. Moreover, we did not find the converse association reported by Steer et al. among the minor allele (GG) homozygotes. Examining dose-response effects, no significant differences in IQ scores of rs174575 C carriers and GG homozy-

TABLE 2 Effect Size and Significance Levels of Feeding Method, Genotypes (rs174575, rs1535, and 174583), and Gene-by-Environment Interaction (G × E) on full-scale IQ (FSIQ) Adjusted for Sex Only and FSIQ Adjusted for Confounders[a]

		Feeding Method			Genotype[b]			G × E[b]		
	n	β	95% CI	p	β	95% CI	p	β	95% CI	p
FSIQ adjusted for sex only										
rs174575	1101	2.5	−1.2 to 4.4	0.12	4.9	−2.4 to 12.1	0.19	1.6	−0.6 to 5.6	0.26
rs1535	1104	2.0	−0.7 to 4.6	0.14	4.2	−1.4 to 9.8	0.14	1.0	−1.2 to 3.2	0.36
rs174583	1104	1.8	−0.8 to 4.4	0.18	3.7	−1.9 to 9.2	0.20	0.9	−1.3 to 3.1	0.44
FSIQ adjusted for confounders										
rs174575	1101	1.4	−1.7 to 4.3	0.38	3.8	−3.2 to 10.6	0.30	0.9	−1.8 to 3.6	0.50
rs1535	1104	1.1	−1.5 to 3.6	0.41	3.4	−2.1 to 8.7	0.23	0.6	−1.5 to 2.7	0.57
rs174583	1104	0.9	−1.6 to 3.4	0.49	2.7	−2.6 to 8.1	0.32	0.5	−1.7 to 2.6	0.68

Note: CI = confidence interval; n = number of individuals for whom genotype and cofounder data were available.

[a] *Sex, socioeconomic status, paternal and maternal education, and birth weight.*

[b] *Test for genotype effects and G × E were carried out under dominant models, C carriers versus GG homozygotes for rs174575 and A carriers versus GG homozygotes for rs1535.*

TABLE 3 Results for Tests of Maternal Genotype Effects, for rs174575, rs1535, and rs174583, on full-scale IQ (FSIQ) Adjusted for Sex and FSIQ Adjusted for All Covariates

		Maternal Genotype								
		rs174575			rs1535			rs174583		
	n	$\Delta\chi^2$	Δdf	p	$\Delta\chi^2$	Δdf	p	$\Delta\chi^2$	Δdf	p
FSIQ adjusted for sex	647	0.10	1	0.75	1.82	1	0.18	0.92	1	0.34
FSIQ adjusted for all covariates	639	0.01	1	0.89	1.33	1	0.24	0.62	1	0.42

Note: n = number of individuals.

gotes were found as a function of duration of breast-feeding.

Among the significant strengths of the present study was its power (>97%), use of additional *FADS2* SNPs, assessment of maternal *FADS2* status, the status of offspring, and access to a comprehensive set of SES and other covariates and measurements of breast-feeding exposure. A notable difference between the samples of Caspi et al. and the present samples is that 80% of children had received some form of breast-feeding, which is likely due to the promotion of breast-feeding by Australian public health authorities in the 1980s and 1990s compared with 50% in the British and New Zealand samples of Caspi et al.[16] The consequence of this high prevalence of breast-feeding (>80%) was a small number of formula-fed GG homozygotes. However, because the effect of *FADS2* status is proposed to be present only in C-allele carriers[16] and we were not looking for a new interaction, having a high prevalence of C-allele carriers (93%) increased our statistical power to replicate the original finding.[16] In addition, previous studies treated breast-feeding as a binary variable,[16,48] whereas we used a duration-based measurement of breast-feeding in the hope that this would be a more sensitive measurement of exposure to fatty acids in breast milk. This was confirmed when our data were reanalyzed treating breast-feeding as a binary variable (breast-fed versus formula-fed), which showed an increase in p value of the interaction of rs174575 status with breast-feeding (p = .50 to .95).

Interestingly, in a recent report Munafo et al.[49] demonstrated that in the absence of a genetic (SNP) main effect or in the presence of a negligible effect, a gene-by-environment interaction involving the same SNP is also likely to have little to no effect. Based on the results of their meta-analysis and simulations, they suggested that reports of gene-by-environment interactions in the absence of a main effect are unlikely to be replicable. Thus, because we found no significant rs174575 main effect on FSIQ, it is probably not surprising that we did not identify a gene-by-environment interaction between rs174575 and breast-feeding. Also, because the original report of Caspi et al.[16] did not find any rs174575 main effect on IQ, the likelihood of replication of the gene-by-environment interaction is further decreased.

Despite our nonreplication, it remains possible that *FADS2* or other genes involved in LC-PUFA metabolism may be potential candidate genes for IQ. The *FADS2* polymorphisms tested in the present study are a fraction of the 99 effective/tag SNPs in the *FADS* complex (comprising 100 kb on each side), potentially affecting fatty acid metabolism in childhood and beyond, and these SNPs remain potential candidates for gene-by-environment interactions. Further investigation of upstream and downstream markers will be necessary to determine if the *FADS* gene cluster has a role in the etiology of cognitive ability.

The significant association reported of maternal education and breast-feeding is in line with previous reports that brighter mothers are more likely to feed their newborn with breast milk rather than with formula and for a longer period.[13] Supporting the meta-analytic findings of Der et al.,[9] we found that although breast-feeding was associated with raw FSIQ in offspring, this association did not remain significant once FSIQ scores were adjusted for sex, SES, paternal and maternal education, and birth weight. Similar findings have been described in another Australian study that used a sample contemporary to ours[15] and in other independent studies that have found little to no significant effect of breast-feeding on IQ after covariate adjustment.[9,10,12,13] Therefore, our results support the hypothesis

that the majority of the association between breast-feeding and intelligence (but perhaps not all forms of cognitive function[50]) reflects variation in parental cognitive ability.[15] The association between paternal education and offspring IQ is also reliably found.[51,52] Given strong assortative mating for education and IQ[53] and moderate to strong genetic transmission of intelligence,[54] the observed association of offspring intelligence with paternal education, SES, and maternal rearing behaviors (all associated with offspring IQ) reflects genetic transmission to a significant degree.

A limitation of our study may be that direct measurements of parental cognitive ability were not available. However, two matters mitigate this as a limitation. First and most importantly, the effect reported by Caspi et al. was unadjusted for maternal IQ. Adjustment decreased rather than increased the magnitude of this effect. Second, use of maternal and paternal education as a proxy for parental IQ provides a high-quality proxy for IQ (e.g., Deary et al.[43]). Another potential limitation of our study was that, in common with other studies in this area, breast-feeding data were collected by retrospective questionnaire and might contain some miss-recollection of breast-feeding duration. Prospective and retrospective reports of breast-feeding (ever versus never) have been shown to be correlated as high as .94 and .86 after 8- and 20-year retrospective assessments, respectively.[55,56] Studies that have investigated maternal recall of breast-feeding duration have found a good reliability with retrospective reports. An Australian study found a difference of 5.2 weeks between longitudinal record and retrospective recall 14 to 15 years later.[57] Similarly, an American study in elderly women 69 to 79 years of age (n = 140) has found that 54% of their participants recalled correctly the duration of breast-feeding and that 89% of them reclassified within plus or minus one category (categories were 1 to 3, 4 to 6, 7 to 9, and 10 to 12 months) 34 to 50 years later.[58]

In conclusion, we did not find significant support for interactions (or main effects) of breast-feeding and *FADS2* genotype on IQ. Instead, our results are compatible with the view that once IQ scores are adjusted for confounding variables, breast-feeding has negligible effects on cognition. We further found that maternal *FADS2* genotypes have no detectable effect on offspring IQ. However, more studies and their subsequent meta-analysis will be required to make a firm conclusion on the relation between *FADS2* and IQ. &

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TABLE S1 Mean (Standard Deviation) for full-scale IQ (FSIQ), Breast-Feeding, and Confounders Socioeconomic Status (SES), Paternal and Maternal Education, Gestational Age, and Birth Weight between Genotyped and Nongenotyped Individuals

Measurements	Genotyped Individuals	Nongenotyped Individuals
Children's FSIQ	113.4 (13)	112.5 (14)
Breast-feeding	2.25 (1.07)	2.41 (1.11)
SES *Z*-score[a]	0.06 (1.0)	0.03 (0.98)
Maternal education	4.26 (2.04)	4.32 (2.07)
Paternal education	4.50 (2.10)	4.58 (2.16)
Gestational age (wk)	37.8 (2.9)	37.2 (3.3)
Birth weight (g)	2,581 (568)	2,472 (586)

Note: Parental education was scored 1 to 8 (ranging from ≤7 years of schooling to university postgraduate training), with all mean values indicating at least 12 years of schooling (score = 4).

[a]*−2.1 = lowest, 2.6 = highest.*

TABLE S2 Mean (Standard Deviation) Comparison of Children Grouped according to Breast-Feeding and Genotypes[a] on Tested Intelligence[b] and Covariates[c]

	Breast-Fed ≥6 mo	Breast-Fed 0-6 mo	Breast-Fed 0-3 mo	Not Breast-Fed
	rs174575 CC Homozygote			
Measures/SNP	**n = 185**	**n = 99**	**n = 208**	**n = 85**
Children's FSIQ	112.8 (12.7)	114.3 (12.5)	113.5 (13.4)	108.6 (12.7)
SES *Z*-score[d]	0.04 (0.92)	0.08 (1.0)	0.17 (0.99)	−0.50 (0.90)
Maternal education	4.47 (2.1)	4.46 (2.2)	4.21 (2.0)	3.45 (1.9)
Paternal education	4.55 (2.2)	4.68 (2.2)	4.54 (2.0)	3.47 (1.8)
Gestational age (wk)	37.7 (2.9)	38.3 (2.6)	37.0 (3.31)	36.4 (3.4)
Birth weight (g)	2,605 (481)	2,682 (533)	2,472 (602)	2,368 (606)
	rs1535 AA Homozygote			
Measures/SNP	**n = 151**	**n = 76**	**n = 162**	**n = 65**
Children's FSIQ	111.9 (12.8)	114.7 (12.3)	112.4 (12.3)	107.3 (13.1)
SES[d]	0.03 (0.90)	0.13 (1.03)	0.13 (0.98)	−0.47 (0.90)
Maternal education	4.50 (2.0)	4.56 (2.1)	4.06 (1.9)	3.41 (1.8)
Paternal education	4.48 (2.1)	4.74 (2.2)	4.46 (2.0)	3.43 (1.7)
Gestational age (wk)	37.7 (2.9)	38.2 (2.6)	37.0 (3.3)	36.6 (3.6)
Birth weight (g)	2,595 (482)	2,665 (498)	2,462 (615)	2,371 (618)
	rs174583 CC Homozygote			
Measures/SNP	**n = 150**	**n = 75**	**n = 159**	**n = 66**
Children's FSIQ	111.8 (12.8)	114.7 (12.2)	112.2 (12.3)	107.3 (13.1)
SES[d]	0.03 (0.90)	0.14 (1.04)	0.12 (0.98)	−0.47 (0.90)
Maternal education	4.48 (2.0)	4.57 (2.1)	3.99 (1.8)	3.41 (1.8)
Paternal education	4.49 (2.1)	4.75 (2.2)	4.45 (2.0)	3.43 (1.7)
Gestational age (wk)	37.7 (2.9)	38.2 (2.6)	37.0 (3.3)	36.6 (3.6)
Birth weight (g)	2,597 (483)	2,673 (497)	2,456 (618)	2,371 (618)

Note: [a]*Single nucleotide polymorphisms (SNP) rs174575, rs1535, and rs174583.*
[b]*Full-scale IQ (FSIQ).*
[c]*Socioeconomic status (SES), paternal and maternal education, gestational age, and birth weight.*
[d]*−2.1 = lowest, 2.6 = highest.*

TABLE S2 Continued

Breast-Fed ≥6 mo	Breast-Fed 0-6 mo	Breast-Fed 0-3 mo	Not Breast-Fed	Breast-Fed ≥6 mo	Breast-Fed 0-6 mo	Breast-Fed 0-3 mo	Not Breast-Fed
	rs174575 CG Heterozygote				rs174575 GG Homozygote		
n = 131	n = 108	n = 140	n = 74	n = 22	n = 12	n = 24	n = 13
113.2 (11.9)	114.0 (12.3)	114.2 (12.5)	107.9 (13.7)	109.8 (10.7)	112.8 (14.4)	115.7 (12.5)	102.3 (10.9)
0.00 (0.96)	0.19 (0.99)	0.20 (0.98)	−0.18 (1.02)	−0.32 (0.83)	0.33 (1.14)	0.23 (1.04)	−0.62 (0.77)
4.19 (1.9)	4.03 (2.0)	4.02 (1.8)	3.45 (1.9)	4.32 (2.3)	5.83 (1.9)	4.69 (2.2)	3.54 (1.6)
4.46 (2.2)	4.56 (2.3)	4.66 (2.0)	4.05 (2.2)	3.77 (2.2)	3.83 (1.7)	4.80 (2.1)	3.38 (1.7)
38.2 (2.1)	37.1 (3.6)	37.6 (3.2)	37.0 (3.1)	37.6 (2.3)	37.5 (3.8)	37.5 (3.0)	39.2 (1.5)
2,722 (490)	2,503 (616)	2,564 (597)	2,457 (544)	2,664 (494)	2,506 (684)	2,677 (610)	2,829 (373)
	rs1535 AG Heterozygote				rs1535 GG Homozygote		
n = 147	n = 128	n = 173	n = 88	n = 41	n = 16	n = 37	n = 20
114.4 (11.9)	113.7 (12.6)	115.6 (13.4)	108.8 (12.8)	110.5 (11.4)	114.71 (13.2)	112.7 (13.0)	104.7 (14.0)
0.04 (0.98)	0.11 (1.00)	0.24 (0.98)	−0.32 (1.03)	−0.23 (0.93)	0.59 (1.08)	0.17 (1.06)	−0.30 (0.81)
4.30 (2.0)	3.94 (2.0)	4.27 (2.0)	3.39 (1.9)	4.07 (2.2)	6.12 (1.9)	4.20 (2.0)	3.85 (1.7)
4.58 (2.3)	4.49 (2.2)	4.66 (2.1)	3.89 (2.2)	4.07 (2.2)	4.44 (2.2)	4.92 (2.1)	3.80 (1.9)
38.1 (2.3)	37.3 (3.5)	37.5 (3.2)	36.7 (3.1)	38.2 (2.2)	37.8 (3.4)	37.4 (3.1)	38.4 (2.4)
2,722 (497)	2,546 (633)	2,571 (587)	2,430 (562)	2,621 (443)	2,507 (610)	2,537 (609)	2,681 (438)
	rs1174583 CT Heterozygote				rs174583 TT Homozygote		
n = 146	n = 127	n = 176	n = 88	n = 43	n = 17	n = 37	n = 20
114.4 (11.9)	113.5 (12.5)	115.7 (13.4)	108.8 (12.8)	111.0 (11.4)	115.6 (13.3)	112.6 (13.0)	104.7 (14.0)
0.05 (0.99)	0.09 (0.99)	0.24 (0.97)	−0.32 (1.03)	−0.22 (0.90)	0.66 (1.08)	0.17 (1.06)	−0.30 (0.81)
4.32 (2.0)	3.95 (2.0)	4.33 (2.0)	3.39 (1.9)	4.07 (2.2)	5.94 (2.0)	4.20 (2.0)	3.85 (1.7)
4.57 (2.3)	4.47 (2.2)	4.68 (2.1)	3.89 (2.2)	4.07 (2.1)	4.59 (2.2)	4.92 (2.1)	3.80 (1.9)
38.1 (2.3)	37.3 (3.5)	37.5 (3.2)	36.7 (3.1)	38.3 (2.2)	37.9 (3.3)	37.4 (3.1)	38.4 (2.4)
2,721 (500)	2,535 (630)	2,575 (583)	2,430 (562)	2,620 (433)	2,555 (626)	2,537 (609)	2,681 (438)

TABLE S3 Results for Tests of Feeding Method Effects, Genotype (rs174575, rs1535, and 174583) Effects, and Gene-by-Environment Interaction (G × E) on full-scale IQ (FSIQ) Adjusted for Sex Only and for Confounders

		Feeding Method			Genotype[a]			G × E[a]		
	n	$\Delta\chi^2$	Δdf	*p*	$\Delta\chi^2$	Δdf	*p*	$\Delta\chi^2$	Δdf	*p*
FSIQ adjusted for sex only										
rs174575	1,101	2.48	1	0.115	1.72	1	0.189	1.27	1	0.260
rs1535	1,104	2.17	1	0.141	2.19	1	0.139	0.83	1	0.363
rs174583	1,104	1.83	1	0.176	1.64	1	0.201	0.61	1	0.436
FSIQ adjusted for confounders[b]										
rs174575	1,101	0.77	1	0.381	1.09	1	0.298	0.45	1	0.504
rs1535	1,104	0.68	1	0.409	1.46	1	0.227	0.33	1	0.567
rs174583	1,104	0.48	1	0.489	1.00	1	0.316	0.18	1	0.668

Note: [a]*Test for genotype effects and G × E effects were carried out under dominant models, C carriers vs. GG homozygotes for rs174575 and A carriers versus GG homozygotes for rs1535.*

[b]*Sex, socioeconomic status, paternal and maternal education, and birth weight.*