Moderation of breastfeeding effects on the IQ by genetic variation in fatty acid metabolism

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Children's intellectual development is influenced by both genetic inheritance and environmental experiences. Breastfeeding is one of the earliest such postnatal experiences. Breastfed children attain higher IQ scores than children not fed breast milk, presumably because of the fatty acids uniquely available in breast milk. Here we show that the association between breastfeeding and IQ is moderated by a genetic variant in *FADS2*, a gene involved in the genetic control of fatty acid pathways. We confirmed this gene–environment interaction in two birth cohorts, and we ruled out alternative explanations of the finding involving gene–exposure correlation, intrauterine growth, social class, and maternal cognitive ability, as well as maternal genotype effects on breastfeeding and breast milk. The finding shows that environmental exposures can be used to uncover novel candidate genes in complex phenotypes. It also shows that genes may work via the environment to shape the IQ, helping to close the nature versus nurture debate.

cognitive development | gene environment interaction

For 100 years, the IQ has been at the heart of scientific and public debates about nature versus nurture (1–3). Twin studies document that differences between individuals' IQs are under strong genetic influence, but twin studies also attest to the existence of nongenetic, environmental influences on IQ, particularly for young children (4, 5). In the past 5 years, the nature versus nurture debate has shifted toward interest in how both nature and nurture work together (6). An integral part of this new focus is research that tests how genetic differences moderate the effects of environmental influences on individuals' health and behavior (7). Here we report replicated evidence that a measured genotype can moderate response to an environmental influence on children's IQ. We began our investigation of gene–environment interaction in IQ by selecting for study an environmental factor thought to influence neurodevelopment and known to predict IQ. We selected being fed breast milk (hereafter breastfeeding) as the environmental exposure because the biological processes underlying its benefits for the developing brain are increasingly well understood (8). A gene involved in these putative biological processes would be a good candidate for framing a gene–environment interaction hypothesis (9). Thus, selecting breastfeeding as the environmental exposure allowed us to nominate a novel candidate gene for this study of IQ.

Breastfeeding is thought to influence brain development through nutritional processes involving fatty acids (10). The predominant long-chain polyunsaturated fatty acids (LC-PUFAs) present in human milk, but not in cow's milk or most infant formulas, are docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (AA or ARA; 20:4n-6) (11). Substantial amounts of DHA and AA accumulate in the human brain during the first postnatal months (12), and infants who are breastfed have higher concentrations of DHA and AA than infants fed unsupplemented formulas (13, 14). Evidence, in general, is consistent with the hypothesis that LC-PUFAs in breast milk may enhance cognitive development (15). In humans, children who are breastfed have higher IQs than children not fed breast milk (16, 17), and this advantage persists into adulthood (17). Although breastfeeding in contemporary, industrialized nations is associated with higher social class, IQ differences between breastfed children and children not fed breast milk remain significant in most observational studies even after adjustments for class-related confounding factors (16, 17). However, the essentiality of fatty acids cannot be inferred from such studies. Experimental studies, where more control can be achieved, show that animals that are fed diets deficient in n-3 fatty acids exhibit neuronal deficits, including memory, sensory, and visual abnormalities (18). DHA supplementation in rodents and nonhuman primates leads to increased brain DHA concentrations and enhanced performance on a wide variety of learning, memory, and problem-solving tasks (19–21). LC-PUFAs are thought to be important for cognitive development because they are required for efficient neurotransmission (22) and are involved in neurite outgrowth, dendritic arborization, and neuron regeneration after cell injury (23). This putative biological pathway led us to search the KEGG database (24) for genes involved in LC-PUFA metabolism, reasoning that they might moderate the effect of breastfeeding on children's IQ.

This search led us to *FADS2*, an attractive candidate gene because of its role in the modification of dietary fatty acids. *FADS2*, located on chromosome 11q12.2, encodes the delta-6 desaturase that is the rate-limiting step on the metabolic pathway leading to AA and DHA production. *FADS2* gene expression is also regulated through end-product inhibition and dietary LC-PUFAs such as those available in breast milk (25). To the best of our knowledge, *FADS2* polymorphisms have not been studied in relation to breast milk or to IQ. As such, we selected two SNPs (rs174575 and rs1535) as candidate biomarkers because they provided the best combination of two desirable factors: (*i*) they had known linkage disequilibrium (LD) throughout the region, and (*ii*) they had minor allele frequencies sufficiently prevalent to permit their use in tests of gene–environment interaction. Specifically, using data from CEPH (Utah residents with ancestry from northern and western Europe) HapMap trios (26), we found that these SNPs showed strong LD throughout the promoter and intragenic region of *FADS2*. In addition, these SNPs exhibited strong LD into the promoter and intragenic region of

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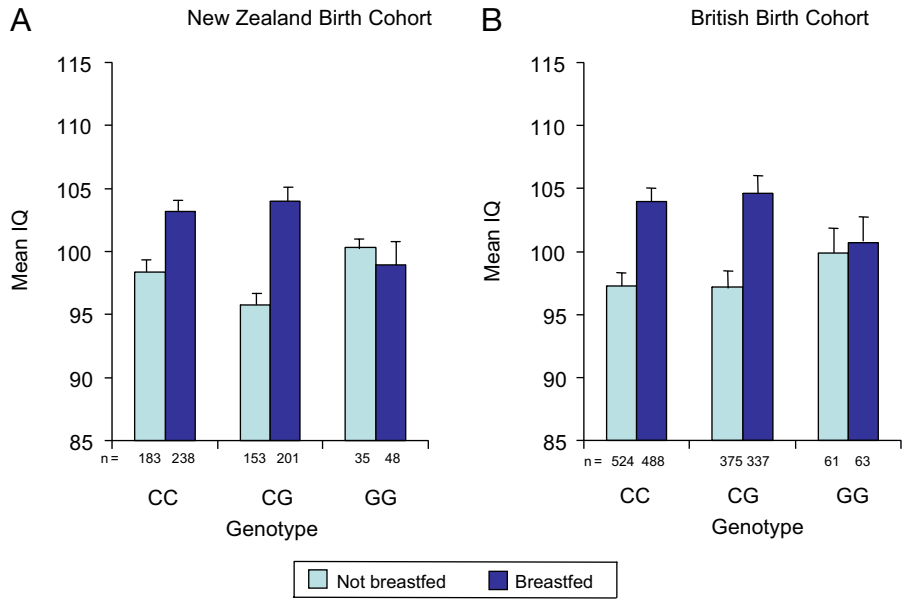

Fig. 1. The association between breastfeeding and IQ is moderated by a genetic polymorphism (rs174575) in the *FADS2* gene. In each cohort, we estimated a hierarchical regression model (ordinary least squares) with main effects for genotype (C carriers vs. GG homozygotes) and environment (not breastfed vs. breastfed) followed by a multiplicative genotype × environment interaction term, with covariate adjustment for socioeconomic status. In the Dunedin cohort (*A*), the effect of breastfeeding was significant ($t = 4.67$, $P < 0.001$), the effect of rs174575 was not significant ($t = 0.32$, $P = 0.75$), and the interaction term was significant ($t = 2.11$, $P = 0.035$). In the E-risk cohort (*B*), the effect of breastfeeding was significant ($t = 3.20$, $P < 0.001$), the effect of rs174575 was not significant ($t = 1.82$, $P = 0.42$), and the interaction term was significant ($t = 2.37$, $P = 0.018$).

FADS1, a highly similar gene that borders the 5′ region of *FADS2* and that is also involved in fatty acid metabolism, encoding the delta-5 desaturase (27). By genotyping these two tag SNPs (identified with the Gabriel method in Haploview v3.2 on release 20 of CEPH HapMap data), we could obtain maximum information about a candidate locus that potentially moderates breastfeeding effects on IQ [see supporting information (SI) *Text* and SI Figs. 3–5 for details about candidate gene and marker selection strategy]. We then tested the hypothesis that the cognitive advantage associated with breastfeeding in humans is related to genetic differences in LC-PUFA metabolism, and we replicated this test in two birth cohorts.

Results

Consistent with previous reports, the difference in IQ test scores between breastfed children and those not breastfed was 5.6 and 6.3 IQ points in the Dunedin and E-risk cohorts, respectively. Genotype was not related to IQ in either cohort. (IQ means associated with the three rs174575 genotype groups, CC, CG, and GG, were 101.1, 100.4, and 99.5 in Dunedin and 100.5, 100.7, and 100.3 in E-risk; IQ means associated with the three rs1535 groups, AA, AG, and GG, were 101.2, 100.3, and 100.9 in Dunedin and 101.0, 100.4, and 99.3 in E-risk.)

Analyses revealed that rs174575 interacted with breastfeeding to influence IQ in both the Dunedin ($P = 0.035$) and E-risk ($P = 0.018$) cohorts (Fig. 1). There was a dominant effect of the C allele in response to breastfeeding. In Dunedin, breastfed children carrying the C allele showed a 6.4-IQ-point advantage relative to children not fed breast milk ($t = 6.35$, $P < 0.001$). In contrast, GG homozygotes neither gained an advantage from breastfeeding nor suffered a disadvantage from not being fed breast milk ($t = 0.50$, $P = 0.62$) (Fig. 1*A*). Turning to the E-risk cohort, we found that breastfed children carrying the C allele showed a 7.0-IQ-point advantage relative to children not fed breast milk ($t = 7.91$, $P < 0.001$), whereas GG homozygotes neither gained an advantage from breastfeeding nor suffered a disadvantage from not being fed breast milk ($t = 0.22$, $P = 0.83$) (Fig. 1*B*).

Four points are relevant for interpreting this replicated gene–environment interaction between rs174575 and breastfeeding in predicting IQ. First, it is important to rule out confounding by social class, because socioeconomic advantage is related to children's higher IQ, and in modern countries, socioeconomically advantaged women are more likely to breastfeed (Table 1). To rule out this potential confound, all significance tests reported here for the rs174575-breastfeeding interaction were conducted with covariate adjustment for social class (see SI Table 2). Second, it is important to rule out confounding by maternal IQ (28). We added statistical controls for measures of maternal cognitive ability (Table 1); the rs174575-breastfeeding interaction remained significant in both Dunedin ($P = 0.03$) and E-risk ($P = 0.03$) (see SI Table 2). Third, to interpret the interaction, it is necessary to rule out potential genotype effects on exposure to breastfeeding. Child genotype was not related to breastfeeding in either cohort; prevalence rates of breastfeeding associated with the three rs174575 genotype groups (CC, CG, and GG) were 56%, 57%, and 58% in Dunedin [χ^2 (2) = 0.10, $P = 0.95$] and 48%, 47%, and 51% in E-risk [χ^2 (2) = 0.30, $P = 0.86$].

Fourth, it is important to rule out potential genotype differences in intrauterine growth. Because small gestational age and lower birth weight have been linked to lower IQ (29, 30), a spurious rs174575-breastfeeding interaction could be produced if GG homozygotes differed from C-carriers in their intrauterine growth. However, there were no significant gestational age or birth weight differences between the genotype groups, in either cohort (Table 1).

We repeated all analyses using rs1535. We observed a significant interaction in the E-risk cohort ($P = 0.01$). Breastfed A-carriers had higher IQs than nonbreastfed A-carriers, whereas this advantage was not as pronounced among GG homozygotes (breastfed children with AA, AG, and GG genotypes had IQs of 104.6, 104.6, and 100.0, whereas nonbreasfted children had IQs of 97.7, 96.8, and 98.6). This interaction did not replicate in the Dunedin cohort ($P = 0.55$); breastfed children with AA, AG, and

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