JECH Online First, published on December 13, 2013 as 10.1136/jech-2013-202500

Breastfeeding duration and cognitive, language and motor development at 18 months of age: Rhea mother–child cohort in Crete, Greece

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jech-2013-202500).

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Received 15 February 2013
Revised 4 November 2013
Accepted 8 November 2013

To cite: Leventakou V, Roumeliotaki T, Koutra K, *et al*. *J Epidemiol Community Health* Published Online First: [*please include* Day Month Year] doi:10.1136/jech-2013-202500

ABSTRACT

Background Breast feeding duration has been associated with improved cognitive development in children. However, few population-based prospective studies have evaluated dose–response relationships of breastfeeding duration with language and motor development at early ages, and results are discrepant.

Methods The study uses data from the prospective mother–child cohort ('Rhea' study) in Crete, Greece. 540 mother–child pairs were included in the present analysis. Information about parental and child characteristics and breastfeeding practices was obtained by interview-administered questionnaires. Trained psychologists assessed cognitive, language and motor development by using the Bayley Scales of Infant Toddler Development (3rd edition) at the age of 18 months.

Results Duration of breast feeding was linearly positively associated with all the Bayley scales, except of gross motor. The association persisted after adjustment for potential confounders with an increase of 0.28 points in the scale of cognitive development (β=0.28; 95% CI 0.01 to 0.55), 0.29 points in the scale of receptive communication (β=0.29; 95% CI 0.04 to 0.54), 0.30 points in the scale of expressive communication (β=0.30; 95% CI 0.04 to 0.57) and 0.29 points in the scale of fine motor development (β=0.29; 95% CI 0.02 to 0.56) per accumulated month of breast feeding. Children who were breast fed longer than 6 months had a 4.44-point increase in the scale of fine motor development (β=4.44; 95% CI 0.06 to 8.82) compared with those never breast fed.

Conclusions Longer duration of breast feeding was associated with increased scores in cognitive, language and motor development at 18 months of age, independently from a wide range of parental and infant characteristics. Additional longitudinal studies and trials are needed to confirm these results.

INTRODUCTION

Breast feeding is a key component of optimal infant nutrition and provides many important health benefits to both babies and mothers. It contains the ideal amounts of fatty acids, lactose, water and amino acids for human digestion, brain development and growth, as well as many bioactive ingredients such as cytokines, nucleotides, hormones and growth factors.[1 2] Its high content of docosahexaenoic acid (DHA; 22:6 n-3), a major form of n-3 long-chain polyunsaturated fatty acids (LC-PUFAs), is well known for its beneficial effects on neurotransmission, neurodevelopment and particularly visual acuity.[3–5] The WHO considers exclusive breast feeding for the first 6 months of life to be the optimal method for feeding infants, a recommendation that is also supported by the American Academy of Paediatrics.[6] Breast feeding is recommended for at least 2 years and for as long thereafter as mother and child desire it. The frequency and duration of breast feeding in Crete, Greece, is relatively low compared with WHO recommendations.[7]

Several recent systematic reviews tried to summarise the effect of breast feeding on child's cognitive development with inconsistent results.[8–10] As both breast feeding and infant neurodevelopment are heavily influenced by socioeconomic and psychosocial factors,[7 11 12] these should be taken into account in studies in order to explain its effects on infant neurodevelopment.

Many of the observational studies in this field have included participants from highly selected groups, such as those born preterm or low-birth-weight infants,[13–16] and it is not clear whether the results of such studies can be extrapolated to the general population. Only three birth cohorts have evaluated neurodevelopment using valid psychometric scales at early ages (<2 years of age).[17–19] One of them reported a significant positive association between breastfeeding duration with gross motor (GM) but not with fine motor (FM) development at the age of 9 months,[17] while a birth cohort from Spain has shown that higher cumulative breast feeding was related with an increase in mental development scores at 14 months, whereas no association was found with psychomotor development scores.[18] The 'Taiwan Birth Cohort Study' has shown that longer duration of breast feeding was positively related to children's GM, FM, language and personal/social development in early childhood (<18 months of age).[19]

The aim of the present study was to investigate the association between breastfeeding practices and child's cognitive, language and motor development at the age of 18 months, as assessed by the 3rd edition of Bayley Scales of Infant and Toddler Development (Bayley-III) in the mother–child cohort, 'Rhea' study in Crete, Greece, after adjusting for several maternal and infant characteristics from pregnancy and throughout the life course of the child.

METHODS

Study population

The 'Rhea' study is a prospective mother–child cohort conducted in Heraklion, Greece.[20] Study

subjects were recruited in early pregnancy at the time of the first major ultrasound examination. The inclusion criteria for study participants were pregnant women, residents in the study area; aged 16 years or above; and no communication handicap. Interview-administered questionnaires were used to obtain information on sociodemographic, environmental and psychosocial factors during pregnancy and early childhood. The study has followed the guidelines of the Declaration of Helsinki. In addition, ethical approval for the study was provided by the ethical committee of the University Hospital in Heraklion, Crete, Greece. Written informed consent was obtained from all women participating in the study.

Of 1765 eligible women, 1610 (91%) agreed to participate and 1388 (86%) were followed up until delivery. A random sample of 828 mothers was contacted by telephone at the 18 months follow-up, and 599 (73%) agreed to participate in the neurodevelopmental assessment.[21 22] We included only women with singleton pregnancies; thus, multiple pregnancies were excluded from the present analysis (n=26). Forty-six women (7.7%) did not provide complete information on breastfeeding practices; therefore, they were excluded from the analysis. We also excluded seven infants due to incomplete examination (n=1), signs of pervasive developmental disorders (PDD) (n=1), plagiocephalus (n=1), brain tumour (n=1), microcephalus (n=2) and hydrocephalus (n=1). Hence, a cohort of 540 (88% of the study population of children with neurodevelopmental assessment) mother–child pairs was available for the present analyses.

Breast feeding

Information on breastfeeding initiation, duration, use of infant formula, complementary food and other kinds of bottle food was collected at the ninth-month postpartum, and this information was updated at the age of 18 months. All mother–child pairs included in this study have provided information at both time points. Mothers were asked if they had ever breast fed their child (or placed the child on their breast to feed). If they never breast fed, the reason was recorded. If women initiated breast feeding, further information on breastfeeding intensity and duration was asked, as well as information regarding the first time they breast fed their infant and the duration of breast feeding.[23] Duration of breast feeding was categorised as 'never' breast fed, breast fed for '1–6 months' (according to WHO recommendations) and breast fed for '>6 months'. Breast feeding was also categorised according to the WHO breastfeeding definitions as exclusive, predominant and complementary breast feeding.[24]

Neurodevelopmental assessment

The children's cognitive, language and motor development was assessed at 18 months (±6 weeks) using Bayley-III.[21 25] Neurodevelopmental assessments were conducted by three trained psychologists, who completed the formal training course in the use, administration and interpretation of Bayley-III. Bayley-III assesses infant and toddler development across three domains: (i) the cognitive scale (COG); (ii) the language scale, which is composed of the receptive communication (RC) and the expressive communication (EC) subtest; and (iii) the motor scale, which is divided into the FM and the GM subtests. All testing was done at the Medical School of the University of Crete and two public hospitals in Heraklion in the presence of the mother. Total administration time was approximately 90 min.

The examiners also noted critical comments about the difficulties or special conditions of the neurodevelopmental assessment so as to evaluate the 'quality of assessment' such as no difficulties, difficulties due to physical problems (eg, physical illness, tiredness, asleep, etc.) and difficulties due to behaviour problems (eg, nervousness, shyness, etc.).

Potential confounders

Potential confounders included characteristics that have an established or potential association with child cognitive, language and motor development and breast feeding, including the following characteristics. (A) *Parental characteristics:* maternal and paternal age at birth; education at recruitment (low level ≤6 years of school, medium level 7–12 years of school and high level university or technical college degree); origin (Greek/non-Greek); maternal working status at 18 months of child's age (yes/no); marital status at birth (married–engaged/other); maternal and paternal smoking during pregnancy (non-smoker/quit during pregnancy/smoker); maternal and paternal smoking at 9 months of child's age (yes/no); parents' relationship at 9 months of child's age (improved/same as before/more arguments); and postpartum depressive symptoms assessed at 8–10 weeks after delivery by using the Edinburgh Postpartum Depression Scale (EPDS).[26] (B) *Perinatal and infant characteristics:* gender (male/female); type of delivery (caesarean/vaginal); siblings at birth (yes/no); birth order (order number); birth weight (g); head circumference (cm); length (cm); gestational age (completed weeks); preterm birth (<37 weeks of gestation; yes/no); neonatal intensive care unit and hospitalisation (yes/no); day care attendance at 18 months of age (yes/no); hours/day spent with mother at 18 months of age (number); hours/day spent with father at 18 months of age (number); and age of introduction of solid foods (months).

Statistical analysis

Neurodevelopmental assessment raw scores were standardised for psychologist and child's age at test administration using a parametric method for the estimation of age-specific reference intervals.[21 27] Standardised residuals were then typified having a mean of 100 points with a 15 SD to homogenise the scales (parameters conventionally used in psychometrics for assessing IQs).

Bivariate associations between normally distributed continuous dependent variables (Bayley scores) and categorical independent variables were studied using either Student t test or ANOVA. Bivariate associations between non-normally continuous exposure variable (breastfeeding duration) and independent variables (predictors) were studied using non-parametric statistical methods (Mann–Whitney, Kruskal–Wallis), whereas associations of categorical exposure variables (breastfeeding initiation, exclusivity of breast feeding, categorised duration) and independent variables (predictors) were tested using Pearson's χ^2 test. Pearson's r or Spearman's rho correlation coefficient was used to estimate the strength of the association between continuous dependent and independent variables.

Multivariable linear regression models were implemented to examine the associations between Bayley scores and any breast feeding (initiation, duration and exclusivity) after adjusting for several confounders. Potential confounders related with the exposure and outcomes of interest in the bivariate models with $p<0.1$ were included in the multivariable models. All multivariable models included 520 mother–child pairs (96.3% of the study population) with available data (non-missing values) on the exposure, outcome and confounding variables. The 'quality

Table 1 Descriptive characteristics of the study population and breastfeeding duration, Rhea cohort study, Crete, Greece (n=540)

		Breastfeeding duration			
	N*	Never (n=60)	1–6 months (n=346)	>6 months (n=134)	p Value†
Parental characteristics					
Maternal age (years), mean (SD)	532	30.5 (5.1)	29.8 (4.7)	31.6 (3.9)	0.001
Paternal age (years), mean (SD)	528	33.8 (6.8)	33.8 (5.2)	35.2 (5.1)	0.032
Maternal education, n (%)					<0.001
Low	65	16 (26.7)	38 (11.2)	11 (8.3)	
Medium	257	33 (55.0)	171 (50.6)	53 (40.2)	
High	207	11 (18.3)	129 (38.2)	68 (51.5)	
Paternal education, n (%)					<0.001
Low	151	29 (49.2)	96 (28.5)	26 (19.8)	
Medium	223	19 (32.2)	155 (46.0)	50 (38.2)	
High	152	11 (18.6)	86 (25.5)	55 (42.0)	
Maternal origin, n (%)					0.864
Greek	517	58 (96.7)	332 (96.5)	128 (95.5)	
Non-Greek	20	2 (3.3)	12 (3.5)	6 (4.5)	
Paternal origin, n (%)					0.395
Greek	518	59 (98.3)	333 (98.2)	127 (96.2)	
Non-Greek	12	1 (1.7)	6 (1.8)	5 (3.8)	
Maternal working status at 18 months, n (%)					0.453
Working	335	27 (45.0)	129 (37.3)	48 (35.8)	
Not working	204	33 (55.0)	217 (62.7)	86 (64.2)	
Marital status, n (%)					0.993
Married/engaged	523	59 (98.3)	335 (98.5)	130 (98.5)	
Other	8	1 (1.7)	5 (1.5)	2 (1.5)	
Maternal smoking during pregnancy, n (%)					<0.001
Non smoker	306	36 (63.2)	199 (59.9)	111 (84.1)	
Quit during pregnancy	117	8 (14.0)	79 (23.8)	12 (9.1)	
Smoker	104	13 (22.8)	54 (16.3)	9 (6.8)	
Maternal smoking postpartum, n (%)					<0.001
Yes	149	27 (45.0)	114 (33.1)	8 (6.0)	
No	387	33 (55.0)	230 (66.9)	125 (94.0)	
Paternal smoking during pregnancy					0.020
Non-smoker	228	23 (40.4)	132 (43.9)	73 (60.8)	
Quit during pregnancy	45	6 (10.5)	32 (10.6)	7 (5.8)	
Smoker	204	28 (49.1)	137 (45.5)	40 (33.3)	
Paternal smoking postpartum, n (%)					0.015
Yes	243	33 (55.0)	164 (47.7)	47 (35.3)	
No	293	27 (45.0)	180 (52.3)	86 (64.7)	
Parents' relationship after birth, n (%)					0.131
Same as before	279	36 (61.0)	177 (51.5)	67 (50.0)	
Improved	155	14 (23.7)	108 (31.4)	33 (24.6)	
More arguments	102	9 (15.3)	59 (17.2)	34 (25.4)	
EPDS, n (%)					0.570
<13	392	45 (88.2)	246 (86.3)	101 (90.2)	
≥13	56	6 (11.8)	39 (13.7)	11 (9.8)	
Perinatal and infant characteristics					
Birth weight (kg); mean (SD)	532	3008.8 (430.2)	3181.0 (427.7)	3270.5 (413.4)	<0.001
Infant gender, n (%)					0.890
Male	290	34 (56.7)	186 (53.8)	71 (53.0)	
Female	249	26 (43.3)	160 (46.2)	63 (47.0)	
Type of delivery, n (%)					0.106
Vaginal	261	22 (36.7)	169 (49.1)	70 (53.0)	
C-section	274	38 (63.3)	175 (50.9)	62 (47.0)	
Siblings at birth, n (%)					0.032
Yes	246	18 (30.0)	167 (48.3)	62 (46.3)	
No	293	42 (70.0)	179 (51.7)	72 (53.7)	
Preterm, n (%)					0.001
Yes	56	14 (23.3)	34 (9.9)	8 (6.0)	
No	479	46 (76.7)	308 (90.1)	126 (94.0)	

Continued

Table 1 Continued

	N*	Breastfeeding duration			p Value†
		Never (n=60)	1–6 months (n=346)	>6 months (n=134)	
Neonatal intensive care unit, n (%)					0.003
Yes	77	16 (27.1)	50 (14.7)	11 (8.2)	
No	456	43 (72.9)	291 (85.3)	123 (91.8)	
Day care attendance at 18 months of age, n (%)					0.972
Yes	51	6 (10.2)	32 (9.3)	13 (9.7)	
No	486	53 (89.8)	313 (90.7)	121 (90.3)	
Introduction of solid foods, n (%)					0.305
<5 months	101	48 (80.0)	274 (79.7)	114 (85.7)	
≥5 months	436	12 (20.0)	70 (20.3)	19 (14.3)	
Hours/day with mother at 18 months of age, mean (SD)	537	9.4 (3.9)	8.5 (3.4)	9.2 (3.4)	0.044
Hours/day with father at 18 months of age, mean (SD)	537	4.0 (2.6)	3.5 (2.3)	3.5 (2.5)	0.429

*Reported frequencies do not include missing values.
†p Values obtained from Pearson's χ^2 tests of independence (for categorical variables) or ANOVA (for continuous measures).
EPDS, Edinburgh Postpartum Depression Scale.

of assessment' and child's gender were treated as a priori confounders and were included in all regression models.

Also, the influence of each confounder on the outcomes was assessed by adding those variables one by one in the models. Sensitivity analyses were performed by excluding (i) preterm and/or low-birth-weight neonates and (ii) women with high levels of postpartum depressive symptoms. Effect modification was evaluated using the likelihood ratio test. Fractional polynomials were used to evaluate the linearity of the relationship between breastfeeding duration and neurodevelopmental outcomes using graphical representation. All hypothesis testing was conducted assuming a 0.05 significance level and a two-sided alternative hypothesis. All statistical analyses were performed using SPSS Statistics 19 software (SPSS Inc, Chicago, Illinois, USA).

RESULTS

The mean duration of any breast feeding was 4.97 (SD 4.38) months and of exclusive breast feeding 0.80 months (SD 1.47). The percentage of non-breastfeeding mothers rises on average by 10% per month, reaching 68% at the sixth month. The prevalence of complementary breast feeding (breast milk and solid or semi-solid foods allowing liquid and non-human milk and formula) was 63% at the first month, dropping to 30% at the sixth month. Approximately 52% of infants were breast fed for 1–6 months (n=249), while 13% of children (n=62) were breast fed for less than 1 month and 35% of children (n=169) were breast fed six or more months postpartum, among breastfed infants (n=480). The prevalence of complementary breast feeding (breast milk and solid or semi-solid foods allowing liquid and non-human milk and formula) was 63% at the first month, dropping to 30% at the sixth month. Only 6% of infants (n=32) were exclusively or predominantly breast fed the first five months of life.

A description of the population characteristics and the distribution of the selected covariates by duration of breast feeding are shown in table 1. Mothers were more likely to breast feed their child longer if they were older, had a university education, did not smoke during pregnancy or after birth. Longer duration of breast feeding was also positively associated with some paternal characteristics: age, higher education and non-smoking during pregnancy or postpartum. Breastfeeding initiation was inversely associated with preterm birth, infant hospitalisation in neonatal care unit and low birth weight. Mothers who participated in the current analysis were significantly older, with higher education, more likely to be married and Greek compared with those who were not included in this analysis from the initial number of women followed until birth (data not shown).

Table 2 presents the crude effect of any breast feeding (initiation and duration) on Bayley-III scales at 18 months of age. Infants who were ever breast fed scored higher in the Bayley-III cognitive, RC and FM scales. Longer duration of any breast feeding (>6 months) was associated with increased scores in cognitive, RC, EC and FM scales. Additional analysis of exclusivity of breast feeding, according to WHO definitions, did not reveal any other significant results (results not shown).

Table 3 presents the adjusted coefficients of the multivariate linear regression analysis between any breast feeding (initiation and duration) and Bayley-III scales at 18 months of age. Infants who were ever breast fed had higher scores in the scales of cognitive, RC and FM development though not statistically significant. Fractional polynomials used to evaluate the form of the relationship did not indicate a significant deviation from linearity (p-gain>0.05 for all scales). Duration of any breast feeding (in months) was positively associated with higher scores in all the Bayley-III developmental scales, except the GM scale. More specifically, per each accumulated month of breast feeding, there was an estimated increase of 0.28 points in the scale of cognitive development (β=0.28; 95% CI 0.01 to 0.55), 0.29-point increase in the scale of RC (β=0.29; 95% CI 0.04 to 0.54), 0.30-point increase in the scale of EC (β=0.30; 95% CI 0.04 to 0.57) and 0.29-point increase in the scale of FM development (β=0.29; 95% CI 0.02 to 0.56) after adjusting for several confounders. Children who were breast fed longer than 6 months had a 4.44-point increase in the scale of FM development (β=4.44; 95% CI 0.06 to 8.82) compared with those never breast fed. Additional analysis with more categories of breastfeeding duration (never, 1–3 months, 4–6 months and >6 months) has shown similar results with the initial analysis. Analysis of exclusivity of breast feeding (exclusive, predominant and complementary) did not show significant differences in the effect estimates for breastfeeding categories (data not shown).

In an additional analysis, we performed a series of models by adding potential confounding variables one by one in an effort to assess the influence of different parental and infant characteristics on the relationship between breastfeeding duration and neurodevelopmental outcomes. The strongest confounder of the

Table 2 Breastfeeding and mean standardised Bayley-III scales at 18 months of age, Rhea cohort study, Crete, Greece (n=540)

			Cognitive			Receptive communication			Expressive communication			Fine motor			Gross motor		
	N	Per cent	Mean	(SD)	p Value*	Mean	(SD)	p Value*	Mean	(SD)	p Value*	Mean	(SD)	p Value*	Mean	(SD)	p Value*
Breast feeding																	
Never	60	11.1	96.8	(13.3)	0.019	96.6	(16.0)	0.035	98.8	(15.0)	0.333	96.2	(13.2)	0.005	99.6	(14.1)	0.530
Ever	480	88.9	101.2	(14.8)		101.2	(14.3)		100.8	(15.1)		101.5	(14.4)		100.8	(15.0)	
Breastfeeding duration																	
Never	60	11.1	96.8	(13.3)	0.003	96.6	(16.0)	0.009	98.8	(15.0)	0.006	96.2	(13.2)	0.010	96.2	(13.2)	0.614
1–6 months	346	64.2	100.2	(14.9)		100.4	(14.4)		99.5	(15.4)		100.9	(14.2)		100.9	(14.2)	
>6 months	134	24.7	104.0	(14.7)		103.4	(13.9)		104.1	(13.8)		103.0	(14.7)		101.7	(15.3)	

*t test and ANOVA were used for differences between continuous normally distributed variables.

Table 3 Multivariable associations between breastfeeding initiation/duration and neurodevelopmental outcomes at age 18 months, Rhea cohort study, Crete, Greece (n=520)

	Cognitive*			Receptive communication*			Expressive communication*			Fine motor*			Gross motor*		
	B	95% CI	p Value	β	95% CI	p Value	β	95% CI	p Value	β	95% CI	p Value	β	95% CI	p Value
Breast feeding															
Never	Ref			Ref			Ref			Ref			Ref		
Ever	1.16	(−2.69 to 5.01)	0.554	0.39	(−3.18 to 3.95)	0.831	−1.64	(−5.44 to 2.17)	0.398	3.33	(−0.50 to 7.17)	0.089	−0.71	(−4.79 to 3.36)	0.731
Breastfeeding duration															
Per month	0.28	(0.01 to 0.55)	0.043	0.29	(0.04 to 0.54)	0.028	0.30	(0.04 to 0.57)	0.026	0.29	(0.02 to 0.56)	0.034	0.06	(−0.23 to 0.35)	0.682
Duration categorised															
Never	Ref			Ref			Ref			Ref			Ref		
1–6 months	0.30	(−3.60 to 4.19)	0.880	−0.35	(−3.96 to 3.27)	0.851	−2.83	(−6.67 to 1.01)	0.148	2.96	(−0.95 to 6.87)	0.137	−0.48	(−4.64 to 3.69)	0.822
>6 months	3.70	(−0.66 to 8.06)	0.096	2.53	(−1.53 to 6.58)	0.221	1.85	(−2.45 to 6.15)	0.398	4.44	(0.06 to 8.82)	0.047	0.33	(−4.33 to 5.00)	0.888

*All models have been adjusted for parental characteristics (maternal and paternal education, maternal and paternal country of origin, maternal age, smoking during pregnancy and working situation) and infant characteristics (birth order, birth weight, gender and quality of assessment).

relationship between breastfeeding duration and neurodevelopmental outcomes was estimated to be maternal education (13% change in effect estimate for cognitive development), followed by maternal working status and parity (<5% decrease for each). No significant interaction was observed between breastfeeding duration and maternal education.

Finally, to elucidate whether prematurity or fetal growth restriction and maternal postpartum depressive symptoms confounded the observed results, we performed additional sensitivity analyses in which we excluded (i) all children who were preterm and/or low-birth-weight neonates (preterm births, n=56; low-birth-weight neonates, n=29) and (ii) all women with high levels of postpartum depressive symptoms (EPDS ≥ 13, n=56). Results did not differ substantially from those derived from the main analysis (see online supplementary tables 1S and 2S).

DISCUSSION

The main finding of this population-based cohort study was the positive and linear association of breastfeeding duration with increased scores in the scales of cognitive, language and FM development at 18 months of age. This is the first longitudinal analysis of breastfeeding practices in association with child cognitive, language and motor development in Greece, a country with relatively low breastfeeding levels.[7 23] The beneficial effect of breast feeding persisted even after adjustment for a large array of potential confounders.

Our findings are consistent with previous studies that have reported positive associations between breast feeding and children's cognitive[8 9 11 18 28–32] and motor development.[17 19 33–37] A recent meta-analysis has shown that ever breast feeding was associated with a 3.2-point increase in the scale of cognitive development compared with formula feeding.[8] Only few population-based studies evaluated dose–response relationships of breastfeeding duration with vocabulary development and verbal comprehension at early childhood,[19 35 36] and all of them have shown positive associations with language skills at the age of 6 months,[35] 18 months[19] and 3 years.[36] As noted in several systematic reviews,[8–10] the great majority of published articles have important methodological issues, making it difficult to fully understand the relationship between breast feeding and child mental development. The main limitations of previous studies are the study design (cross-sectional), and data quality (as a consequence of retrospective data collection on breast feeding at the time of cognitive measurements), lack of valid neuropsychological measurements, small sample sizes and insufficient adjustment for critical potential confounders.

There are several potential mechanisms linking breast feeding with enhanced neurodevelopment in infancy. Some studies have concentrated on the effects of particular components of breast milk, although it is not clear which constituent(s) of breast milk might be most beneficial in promoting brain development. Most of them indicated the beneficial role of LC-PUFAs, which are structural lipids critical for retina and cortical brain development in early life.[38] However, randomised trials with infant formulas supplemented with LC-PUFAs have generally not found any clear effects.[39 40] Infant levels of LC-PUFAs depend not only on maternal levels but also on genes such as FADS1 and FADS2 regulating PUFA metabolism and determining PUFA levels in breast milk.[41 42] Recent publications have revealed that genetic variation of FADS genes may modify the effect of breast feeding on cognitive development.[43] Breast milk also contains hormones, oligosaccharides, phospholipids and other trophic factors that are important for optimal neural function.[44] Apart from nutrient components of human milk, there are other possible mechanisms that may explain the association between breast feeding and child neurodevelopment. Breast feeding provides enhanced psychosocial experiences for children such as mother–child interaction, bonding and greater variety of daily stimulations that may contribute to the development of the infant's limbic system and its cortical connections.[45 46] In the present study, longer duration of breast feeding was still positively correlated with young children's cognitive, language and motor development after adjusting for maternal education, employment and other confounding factors.

Strengths of the present study include the population-based prospective design, the high participation rate (72%) and the assessment of a number of potential predictors of child neurodevelopment. Most of the studies evaluating breast feeding in association with cognitive development compared only the cognitive function between 'never breastfed' and 'ever breastfed' children and were not able to provide estimates of the effects of long-term breast feeding on child neurodevelopment.[10] However, in the present analysis, we have measured breast feeding primarily as a continuous variable in order to avoid misclassification bias, to maximise statistical power and to allow detection of dose–response relationships. Bayley-III, which was used for the neurodevelopmental assessment of children, is recognised internationally as one of the most comprehensive tools to identify infant and toddler strengths and competencies, as well as their weaknesses, and to provide a valid and reliable measure of a child's cognitive, language and motor abilities.[25] Deviant language development, neurocognitive functioning and impaired social interaction in the first 18 months of life, as assessed by the Bayley scales among other instruments, can predict the child having any neurodevelopmental disorder at a later age.[47] Participants were unaware of the hypothesis being tested, so misclassification of breastfeeding practices estimated by the questionnaire is likely to be random with respect to neurodevelopmental outcomes. Although the study sample in the 'Rhea' cohort included pregnant women who visited the two local public hospitals, as well as the two major local private maternity clinics, we need to consider significant differences in the population studied compared with participants not included in the present analyses when considering generalisability of results.

Several limitations need to be taken into account. We did not have data on biomarkers of environmental contaminants in breast milk, such as methyl mercury or polychlorinated biphenyls, both of which may harm child development.[48] However, preliminary results from the Rhea study have shown that prenatal concentrations of polychlorinated biphenyls as measured in maternal blood are extremely low in this population compared with other mother–child cohorts in Europe.[49] The possibility of residual confounding cannot be fully discounted since the inclusion of maternal and paternal educational level and maternal working status in the multivariate models might not have removed part of the variance in maternal intelligence, variable not available in the present analysis. Moreover, we had no information on the quality of home stimulation that has been shown as a positive predictor on neurodevelopmental outcomes in early childhood. Exposure information relies on a recall over a 9-month interval, and it could introduce recall bias on breastfeeding practices. Another limitation of this study may be that neurodevelopmental testing was performed at a single time point (18 months of age). Longitudinal data related also to children's neurodevelopment at 4 years of age will be available for analysis in due course and will help us to examine whether protective effects of breast feeding are sustained into early childhood as well. This is the first analysis of breastfeeding practices in association with child neurodevelopment in Greece indicating

the association of longer duration of breast feeding during the first year of life with higher neurodevelopmental scores at 18 months. The follow-up of this birth cohort will allow us to explore if this beneficial effect of breast feeding persists at older ages.

What is already known on this subject

- Breastfeeding duration has been associated with improved cognitive development in children.
- Few population-based prospective studies have evaluated dose–response relationships of breastfeeding duration with communication skills and motor development before the age of 2 years, and results are discrepant.

What this study adds

- The study has shown a positive and linear association of breastfeeding duration with increased scores in the scales of cognitive, language and fine motor development at 18 months of age.
- Further follow-up of this population-based birth cohort will allow us to explore if this beneficial effect of breast feeding persists at older ages.

Acknowledgements The authors would especially like to thank all the cohort participants for their generous collaboration.

Contributors All authors had a substantial contribution to the study, and they have all reviewed and approved the submitted manuscript VL participated in the field study coordination, participated in the statistical analysis and wrote the first draft of the paper. TR supervised the statistical analysis and helped with the data interpretation and manuscript preparation. KK supervised the neurodevelopmental assessment and contributed to the drafting of the manuscript. MV provided feedback and critical revision of the manuscript and helped with data interpretation. EM and PB participated in the design of the study and in the drafting of the paper. MK and LC conceived the study, supervised the data collection, provided critical review of the manuscript and helped with data interpretation and manuscript preparation.

Funding Rhea project was financially supported by European projects (EU FP6-2003-Food-CT-016320-2 NewGeneris, EU FP6. STREP Contract No 36224 Hiwate, EU FP7 ENV.2007.1.2.2.2. Contract No 211250 Escape, EU FP7-2008-ENV-1.2.1.4 Contract No. 226756 Envirogenomarkers, EU FP7-HEALTH-2009-single-stage Contract No. 241604 CHICOS, EU FP7 ENV.2008.1.2.1.6. Contract No 226285 ENRIECO) and the Greek Ministry of Health (Program of Prevention and early diagnosis of Obesity and Neurodevelopmental Disorders in Preschool Children in Heraklion district, Crete, Greece: 2011–2014 NSRF 2007–2013 project, MIS 349580, co-financed by the European Union-European Social Fund and the Hellenic Ministry of Health).

Competing interests None.

Ethics approval The Ethical Committee of the University Hospital in Heraklion, Crete, Greece.

Provenance and peer review Not commissioned; externally peer reviewed.

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