Folic Acid Supplements in Pregnancy and Severe Language Delay in Children

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Context Prenatal folic acid supplements reduce the risk of neural tube defects and may have beneficial effects on other aspects of neurodevelopment.

Objective To examine associations between mothers' use of prenatal folic acid supplements and risk of severe language delay in their children at age 3 years.

Design, Setting, and Patients The prospective observational Norwegian Mother and Child Cohort Study recruited pregnant women between 1999 and December 2008. Data on children born before 2008 whose mothers returned the 3-year follow-up questionnaire by June 16, 2010, were used. Maternal use of folic acid supplements within the interval from 4 weeks before to 8 weeks after conception was the exposure. Relative risks were approximated by estimating odds ratios (ORs) with 95% CIs in a logistic regression analysis.

Main Outcome Measure Children's language competency at age 3 years measured by maternal report on a 6-point ordinal language grammar scale. Children with minimal expressive language (only 1-word or unintelligible utterances) were rated as having severe language delay.

Results Among 38 954 children, 204 (0.5%) had severe language delay. Children whose mothers took no dietary supplements in the specified exposure interval were the reference group (n=9052 [24.0%], with severe language delay in 81 children [0.9%]). Adjusted ORs for 3 patterns of exposure to maternal dietary supplements were (1) other supplements, but no folic acid (n=2480 [6.6%], with severe language delay in 22 children [0.9%]; OR, 1.04; 95% CI, 0.62-1.74); (2) folic acid only (n=7127 [18.9%], with severe language delay in 28 children [0.4%]; OR, 0.55; 95% CI, 0.35-0.86); and (3) folic acid in combination with other supplements (n=19 005 [50.5%], with severe language delay in 73 children [0.4%]; OR, 0.55; 95% CI, 0.39-0.78).

Conclusion Among this Norwegian cohort of mothers and children, maternal use of folic acid supplements in early pregnancy was associated with a reduced risk of severe language delay in children at age 3 years.

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RANDOMIZED CONTROLLED trials and other studies have demonstrated that periconceptional folic acid supplements reduce the risk of neural tube defects.[1-5] To our knowledge, none of the trials have followed up their sample to investigate whether these supplements have effects on neurodevelopment that are only manifest after birth. Animal and some human studies have shown the importance of folate for cell proliferation,[6] central nervous system cell repair,[7] and appropriate epigenetic expression of the genome.[8,9] Thus, folic acid supplement use in relation to child neurodevelopment requires investigation.

We used the prospective Norwegian Mother and Child Cohort Study (Norwegian: Den norske mor & barn-undersøkelsen [MoBa]) to investigate whether maternal use of folic acid supplements was associated with a reduced risk of severe language delay among offspring. Unlike the United States, Norway does not fortify foods with folic acid, increasing the contrast in relative folate status between women who do and do not take folic acid supplements.

METHODS

Study Population

MoBa is a prospective pregnancy cohort that has been described in detail elsewhere.[10] Pregnant women from Norway were recruited to the study through a postal invitation in connection with the routine ultrasound examination offered to all pregnant women at their local hospital around gestational week 17. During the period of recruitment between 1999 and December 2008, 108 841 pregnant women enrolled in the study, with a participation rate of 38.5% (http://www.fhi.no/moba-en). Written informed consent was obtained from each participant

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and the study was approved by the Regional Committee for Medical Research and the Norwegian Data Inspectorate.

The data collection during pregnancy and at birth included self-report questionnaires and biological samples from the mother, father, and child. Follow-up after birth is ongoing and designed to be long term. Up to age 3 years, follow-up included questionnaires periodically sent to mothers for the entire sample. We used the quality ensured Data Version 5 released by MoBa in 2010. Included in this study were 44 220 children born before 2008 for whom the age 3 years questionnaire had been returned by the mother by June 16, 2010, and processed for inclusion in Data Version 5. All questions used in MoBa can be found online at http://www.fhi.no/moba-en.

Measure of Severe Language Delay

The present analysis of age 3 years outcomes focuses on severe language delay. Although a rare outcome, it has clinical significance and is associated with a range of neurodevelopmental disorders.[11] In a corollary analysis, we also examined moderate language delay at age 3 years, an outcome that is less readily interpretable, because many children with moderate language delay will later catch up with their peers.[12]

On a language grammar rating scale[13] in the age 3 years questionnaire, the mother was asked to choose 1 of 6 categories, ranging from no word production to full sentences with complete grammatical markings. Children whose mothers reported minimal expressive language (only 1-word or unintelligible utterances) were rated as having severe language delay. Children who could only produce 2- to 3-word phrases, such as "Me got ball," were rated as having moderate language delay. The remaining children were producing fairly complete sentences, or long and complicated sentences, and were rated as having no language delay. Children with no word production were excluded.

Parental self-report is generally a good measure of early expressive vocabulary, especially for severe language delay.[13,14] To check whether this applied within our study sample, we used a subsample of 425 children. These children were administered in-depth assessments as part of an ongoing case-control study of autism spectrum disorders nested within the MoBa cohort,[15] in which screen-positive potential cases and control children are assessed shortly after completion of the age 3 years questionnaire. We compared scores on the Vineland communication domain[16] with ratings based on maternal report in the age 3 years questionnaire. The Vineland, a semistructured interview, was administered by clinicians who were blind to the maternal reports. The communication domain evaluates the child's receptive and expressive communication skills.[16]

Measures of Motor Delay

We examined delay in gross motor skills as a secondary outcome. If prenatal use of folic acid supplements exhibited an association with severe delay in language but not gross motor skills, it would suggest some specificity to the association. The MoBa questionnaire at age 3 years included 2 age-specific questions on attainment of gross motor skills drawn from the Ages and Stages Questionnaires[17] ("Can your child kick a ball by swinging his/her leg forward without holding onto anything for support?" and "Can your child catch a large ball with both hands?"). Mothers could respond yes, sometimes, or not yet. Children were rated as having significant delay in gross motor skills if the mother reported that they had "not yet" attained either of these gross motor skills.

To provide a broader picture of neurodevelopment in the children with severe language delay, we also examined 6 questions at age 18 months and 4 questions at age 3 years that pertained to motor development. We compared children with severe language delay, moderate language delay, and no language delay with respect to the proportion whose mothers reported that they had "not yet" attained each motor skill.

Measure of Folic Acid Use

The women received a questionnaire in week 17 of pregnancy with detailed questions about use of vitamins, minerals, and other dietary supplements in 4-week time windows from before conception. They were asked to record use according to the ingredient list on the supplement container. Previous studies have described use of folic acid and other dietary supplements in this cohort.[18,19]

Because early gestation is a sensitive period for some mechanisms by which folic acid might affect neurodevelopment,[20,21] we focused on folic acid supplements in early rather than late gestation. We could not, however, precisely demarcate the relevant window of exposure. Therefore, we first tested the a priori hypothesis that folic acid supplement use from 4 weeks before to 8 weeks after conception would be associated with reduced risk of severe language delay in children, and then explored the other windows of exposure. We defined 4 mutually exclusive categories of dietary supplement use within the period from 4 weeks before to 8 weeks after conception: (1) no use of dietary supplements; (2) other supplements, but no folic acid; (3) folic acid only; and (4) folic acid in combination with other supplements. No use of dietary supplements was chosen as the reference group, because comparisons to this group would be most readily interpretable and would be relatively precise due to its large size. A potential disadvantage of choosing this reference group is that women who did not use any supplements before gestational week 8 might differ on unknown confounders from women in other groups.

In some of our exploratory analyses, due to small numbers in these 4 exposure groups, we collapsed the 4 categories described above into no use of folic acid (combines 1 and 2 above) and use of folic acid (combines 3 and 4 above). This was done in our analysis of the relevant period of exposure and in stratified analyses conducted within each level of maternal education.

Potential Confounders

We considered numerous factors that might be associated with use of folic acid supplements and with severe language delay in the child. As shown in TABLE 1, these included maternal level of education, paternal level of education, maternal age, paternal age,

Table 1. Parental Characteristics by Maternal Use of Supplements From 4 Weeks Before to 8 Weeks After Conception[a]

Characteristics	No. (%)				
	Total (N = 38 954)	No Supplements (n = 9460)	Other Supplements, No Folic Acid (n = 2586)	Folic Acid Only (n = 7354)	Folic Acid Plus Other Supplements (n = 19 554)
Maternal education, y					
<12	6671 (17.1)	2510 (26.5)	546 (21.1)	1194 (16.2)	2421 (12.4)
12	4656 (12.0)	1484 (15.7)	397 (15.4)	883 (12.0)	1892 (9.7)
13-16	17 189 (44.1)	3668 (38.8)	1052 (40.7)	3426 (46.6)	9043 (46.2)
≥17	9576 (24.6)	1526 (16.1)	526 (20.3)	1688 (23.0)	5836 (29.8)
Missing data	862 (2.2)	272 (2.9)	65 (2.5)	163 (2.2)	362 (1.9)
Paternal education, y					
<12	12 845 (33.0)	4001 (42.3)	974 (37.7)	2447 (33.3)	5423 (33.0)
12	3894 (10.0)	1041 (11.0)	267 (10.3)	780 (10.3)	1806 (10.0)
13-16	10 879 (27.9)	2226 (23.5)	689 (26.6)	2 104 (26.6)	5860 (27.9)
≥17	8951 (23.0)	1488 (15.7)	486 (18.8)	1612 (18.8)	5365 (23.0)
Missing data	2385 (6.1)	704 (7.4)	170 (6.6)	411 (6.6)	1100 (6.1)
Maternal age, y					
<25	3741 (9.6)	1228 (13.0)	290 (11.2)	664 (9.0)	1559 (8.0)
25-29	13 140 (33.7)	3087 (32.6)	759 (29.4)	2622 (35.7)	6672 (34.1)
30-34	15 423 (39.6)	3459 (36.6)	990 (38.3)	2963 (40.3)	8011 (41.0)
≥35	6650 (17.1)	1686 (17.8)	547 (21.2)	1105 (15.0)	3312 (16.9)
Paternal age, y					
<25	1537 (3.9)	541 (5.7)	128 (4.9)	271 (3.7)	597 (3.1)
25-29	8774 (22.5)	2096 (22.2)	526 (20.3)	1681 (22.9)	4471 (22.9)
30-34	15 632 (40.1)	3570 (37.7)	950 (36.7)	3037 (41.3)	8075 (41.3)
35-39	9122 (23.4)	2220 (23.5)	653 (25.3)	1672 (22.7)	4577 (23.4)
≥40	3809 (9.8)	1014 (10.7)	325 (12.6)	684 (9.3)	1786 (9.1)
Missing data	80 (0.2)	19 (0.2)	4 (0.2)	9 (0.1)	48 (0.2)
Planned pregnancy					
No	6674 (17.1)	2131 (22.5)	590 (22.8)	987 (13.4)	2966 (15.2)
Yes	31 852 (81.8)	7204 (76.2)	1957 (75.7)	6288 (85.5)	16 403 (83.9)
Missing data	428 (1.1)	125 (1.3)	39 (1.5)	79 (1.1)	185 (0.9)
Maternal smoking[b]					
No	35 840 (92.0)	8193 (86.6)	2323 (89.8)	6858 (93.3)	18 466 (94.4)
Yes	2840 (7.3)	1163 (12.3)	237 (9.2)	454 (6.2)	986 (5.0)
Missing data	274 (0.7)	104 (1.1)	26 (1.0)	42 (0.6)	102 (0.5)
Maternal alcohol intake[c]					
No	30 664 (78.7)	7206 (76.2)	1920 (74.2)	5857 (79.6)	15 681 (80.2)
Yes	4812 (12.4)	1296 (13.7)	405 (15.7)	827 (11.2)	2284 (11.7)
Missing data	3478 (8.9)	958 (10.1)	261 (10.1)	670 (9.1)	1589 (8.1)
Maternal BMI					
<25	26 211 (67.3)	5908 (62.5)	1776 (68.7)	4789 (65.1)	13 738 (70.3)
25-29	8354 (21.4)	2205 (23.3)	546 (21.1)	1701 (23.1)	3902 (20.0)
30-34	2549 (6.5)	781 (8.3)	146 (5.6)	505 (6.9)	1117 (5.7)
≥35	905 (2.3)	270 (2.9)	58 (2.2)	178 (2.4)	399 (2.0)
Missing data	935 (2.4)	296 (3.1)	60 (2.3)	181 (2.5)	398 (2.0)
Parity[d]					
0	18 575 (47.7)	3889 (41.1)	1142 (44.2)	3314 (45.1)	10 230 (52.3)
1	13 674 (35.1)	3349 (35.4)	848 (32.8)	2848 (38.7)	6629 (33.9)
≥2	6705 (17.2)	2222 (23.5)	596 (23.0)	1192 (16.2)	2695 (13.8)
Maternal height, m					
<1.65	9910 (25.4)	2619 (27.7)	660 (25.5)	1870 (25.4)	4761 (24.3)
1.65-1.68	10 008 (25.7)	2377 (25.1)	678 (26.2)	1903 (25.9)	5050 (25.8)
1.69-1.72	9518 (24.4)	2227 (23.5)	614 (23.7)	1799 (24.5)	4878 (24.9)
>1.72	9115 (23.4)	2115 (22.4)	609 (23.5)	1697 (23.1)	4694 (24.0)
Missing data	403 (1.0)	122 (1.3)	25 (1.0)	85 (1.2)	171 (0.9)

(continued)

Table 1. Parental Characteristics by Maternal Use of Supplements From 4 Weeks Before to 8 Weeks After Conception[a] (continued)

Characteristics	No. (%)				
	Total (N = 38 954)	No Supplements (n = 9460)	Other Supplements, No Folic Acid (n = 2586)	Folic Acid Only (n = 7354)	Folic Acid Plus Other Supplements (n = 19 554)
Marital status[e]					
Married or living with partner	36 214 (93.0)	8584 (90.7)	2349 (90.8)	6882 (93.6)	18 399 (94.1)
Single	1150 (3.0)	401 (4.2)	101 (3.9)	168 (2.3)	480 (2.5)
Other	675 (1.7)	189 (2.0)	48 (1.9)	124 (1.7)	314 (1.6)
Missing data	915 (2.3)	286 (3.0)	88 (3.4)	180 (2.4)	361 (1.8)
Infant nutrition at 6 mo					
Breast milk	22 588 (58.0)	5129 (54.2)	1523 (58.9)	4249 (57.8)	11 687 (59.8)
Infant formula	4844 (12.4)	1518 (16.0)	315 (12.2)	904 (12.3)	2107 (10.8)
Breast milk and infant formula	7693 (19.7)	1785 (18.9)	480 (18.6)	1461 (19.9)	3967 (20.3)
Missing data	3829 (9.8)	1028 (10.9)	268 (10.4)	740 (10.1)	1793 (9.2)

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared).
[a] $P < .001$ for all variables, 2-sided χ^2 test of independence.
[b] Maternal smoking, daily or sometimes, in this pregnancy.
[c] Use of 1 or more alcoholic units per week in the first trimester. One unit equals 1.5 cL of pure alcohol.
[d] Parity including abortion after week 22.
[e] Marital status from age 3 years questionnaire.

whether the pregnancy was planned, maternal smoking in pregnancy, alcohol use in the first trimester, prepregnancy body mass index (BMI, calculated as weight in kilograms divided by height in meters squared), parity, maternal height in meters, marital status, and breastfeeding at 6 months.

Statistical Analyses

The study was adequately powered to detect moderate or large differences in the risk of severe language delay at age 3 years between children unexposed and exposed to maternal use of folic acid. We had good estimates of the frequencies of exposure and outcome from data collected in the early years of the MoBa study. With type I error set at $\alpha = .05$ (2-sided), we computed the sample size that would be required to detect a range of odds ratios (ORs) with 70%, 80%, or 90% power. For the purpose of illustration, using the frequencies actually observed in the present study (60% for use of folic acid supplements and 0.5% for language delay), which are similar to estimates based on previous data, a sample size of 30 000 would be required to detect an OR of 0.5 with 90% power.

We reported the results of 2-sided χ^2 tests for independence between exposure and potential confounders (Table 1) and between outcome and potential confounders (eTable 1, available at http://www.jama.com) analyzed using SPSS version 17.0 (SPSS Inc, Chicago, Illinois). Relative risks for severe language delay were approximated by estimating ORs with 95% CIs using logistic regression models. We used generalized estimating equations, with logit link function and exchangeable correlation, to correct for possible correlations between siblings (>1 pregnancy per participating woman). STATA version 9.0 (StataCorp, College Station, Texas) was used for fitting the regression models. To assess the stability of the association between folate and severe language delay in our main analysis, we also applied 5 alternative analytical approaches.

RESULTS

Age 3 years questionnaires were returned by the mother for 44 220 children (61%). For our primary analyses, to isolate folic acid supplement exposure from other factors, we excluded twins and triplets (n = 1809), children born before gestational week 32 (n = 1847), children with birth weight of less than 2.5 kg (n = 2217), and children reported by their mothers to have hearing problems (n = 1561), resulting in 4950 children (some children were included in more than 1 category). In addition, we excluded 253 children with missing data on the language measure. We also excluded a small heterogeneous group of 63 children who had no word production; a majority of them had various chromosomal abnormalities or other severe syndromes. This resulted in 38 954 children for our main analysis (19 956 boys and 18 998 girls). Of these 38 954 children, 204 (0.5%) were rated as having severe language delay (159 [0.8%] boys and 45 [0.2%] girls), 1290 (3.3%) as having moderate delay (941 [4.7%] boys and 349 [1.8%] girls), and 37 460 (96.2%) as having no language delay (18 856 [94.5%] boys and 18 604 [97.9%] girls).

We first examined the relationship between severe language delay in children at age 3 years and maternal use of dietary supplements in the period 4 weeks before to 8 weeks after conception. Among the potential confounders, maternal education was most strongly associated with both exposure (Table 1) and outcome (eTable 1). Parity, BMI, and marital status also had notable associations with both no use of dietary supplements and severe language delay. We therefore present results unadjusted and adjusted for maternal education, parity, maternal BMI, and marital status (TABLE 2). Missing data were excluded list-wise in these analyses, because there were few miss-

ing data (862 missing maternal education, 935 missing maternal BMI, 915 missing marital status, and 0 missing parity). Compared with the group with no use of dietary supplements, the adjusted ORs were 1.04 (95% CI, 0.62-1.74) for other supplements, but no folic acid; 0.55 (95% CI, 0.35-0.86) for folic acid only; and 0.55 (95% CI, 0.39-0.78) for folic acid in combination with other supplements. These ORs were similar for boys and girls examined separately (the number of girls was small and the CI wide) (eTable 2).

Table 2 shows the results of our exploratory analysis of the relevant period of exposure for severe language delay. Of the women who did not use any supplements in the period 4 weeks before to 8 weeks after conception, 39.1% had started doing so by week 13 to week 17 of pregnancy. Compared with women who did not use any folic acid supplement up to week 17, the risk of severe language delay was lower in children of women who initiated folic acid supplement use in any period before week 8. It was not lower in children of women who initiated use after week 8; we caution that this null result was based on a smaller number of women.

We verified that the main results (Table 2) did not change under 5 alternative analytic approaches: (1) adjusting for all potential confounders (eTable 3A); (2) including specific categories for missing data in this fully adjusted model (eTable 3B); (3) using other supplements, but no folic acid as the reference group (eTable 4); (4) including children born before gestational week 32, with birth weight of less than 2.5 kg, and/or with hearing problems (eTable 5); or (5) including children with no word production in the severe language delay group (eTable 6). We also confirmed that the association between exposure and outcome was in the same direction within each level of maternal education (eTable 7). We addressed the potential for selection bias to influence our results by examining whether there was a synergistic effect of maternal use of folic acid and maternal education on the probability of mothers returning the age 3 years questionnaire; no synergistic effect was found (eTable 8).

We conducted a corollary analysis of the relationship between maternal use of dietary supplements and moderate language delay in children at age 3 years

Table 2. Risk of Having a Child With Severe Language Delay According to Use of Maternal Folic Acid Supplements

	No. (%) of Children		Odds Ratio (95% CI)	
	Overall (n = 37 664)[a]	With Severe Language Delay	Unadjusted (n = 37 664)[a]	Adjusted (n = 35 135)[b]
Supplement use (4 wk before to 8 wk after conception)				
None	9052 (24.0)	81 (0.9)	1 [Reference]	1 [Reference]
Other supplements, no folic acid	2480 (6.6)	22 (0.9)	0.99 (0.61-1.59)	1.04 (0.62-1.74)
Folic acid only	7127 (18.9)	28 (0.4)	0.43 (0.28-0.67)	0.55 (0.35-0.86)
Folic acid plus other supplements	19 005 (50.5)	73 (0.4)	0.42 (0.31-0.58)	0.55 (0.39-0.78)
Initiation of folic acid (4 wk before to 17 wk after conception)[c]				
None	6832 (18.1)	63 (0.9)	1 [Reference]	1 [Reference]
Week −4 to −1	12 208 (32.4)	39 (0.3)	0.34 (0.23-0.51)	0.48 (0.31-0.74)
Week 0 to 4	6819 (18.1)	33 (0.5)	0.52 (0.34-0.79)	0.67 (0.42-1.06)
Week 5 to 8	7105 (18.9)	29 (0.4)	0.44 (0.28-0.68)	0.64 (0.40-1.02)
Week 9 to 12	2547 (6.8)	22 (0.8)	0.93 (0.57-1.52)	1.13 (0.66-1.92)
Week 13 to 17	2153 (5.7)	18 (0.8)	0.90 (0.53-1.53)	1.18 (0.68-2.05)

[a] Excluding moderate language delay (n = 1290).
[b] Adjusted for maternal education, maternal body mass index, parity, and marital status. Moderate language delay (n = 1290) and missing confounder data (n = 2529) excluded from analysis.
[c] The 4 exposure categories in supplement use collapsed into "no/yes," with "no" indicating no use of folic acid (no supplements and other supplements, no folic acid) and "yes" indicating use of folic acid (folic acid only and folic acid plus other supplements). No use of folic acid in the period 4 weeks before to 17 weeks after conception is the reference group of use of folic acid in 5 intervals, with weeks −4 to 17 defining the 5 exposed groups.

Table 3. Risk of Having a Child With Moderate Language Delay According to Use of Maternal Folic Acid Supplements

	No. (%) of Children		Odds Ratio (95% CI)	
Supplement Use (4 wk Before to 8 wk After Conception)	Overall (n = 38 750)[a]	With Moderate Language Delay	Unadjusted (n = 38 750)[a]	Adjusted (n = 36 136)[b]
None	9379 (24.2)	408 (4.4)	1 [Reference]	1 [Reference]
Other supplements, no folic acid	2564 (6.6)	106 (4.1)	0.94 (0.76-1.18)	1.04 (0.83-1.30)
Folic acid only	7326 (18.9)	227 (3.1)	0.70 (0.59-0.82)	0.82 (0.69-0.97)
Folic acid plus other supplements	19 481 (50.3)	549 (2.8)	0.63 (0.56-0.72)	0.79 (0.68-0.90)

[a] Excluding severe language delay (n = 204).
[b] Adjusted for maternal education, maternal body mass index, parity, and marital status. Severe language delay (n = 204) and missing confounder data (n = 2614) excluded from analysis.

Table 4. Association Between Motor Development at 18 Months and at 3 Years and Language Delay

Children Who Had Not Yet Attained Specific Motor Skills	No. (%) of Children			
	Total	No Language Delay	Moderate Language Delay	Severe Language Delay
At 18 mo (n = 34 829)[a]				
Walks rather than crawls	356 (1.0)	276 (0.8)	49 (4.3)	31 (17.7)
Walks well, seldom falls	619 (1.8)	503 (1.5)	77 (6.8)	39 (22.3)
Walks stairs when hand held	2352 (6.8)	2123 (6.3)	179 (15.7)	50 (28.6)
Throws a small ball with forward arm movement	2616 (7.5)	2431 (7.3)	143 (12.5)	42 (24.0)
Stacks small blocks on top of another	983 (2.8)	883 (2.6)	69 (6.1)	31 (17.7)
Turn pages in book by himself/herself	73 (0.2)	51 (0.2)	11 (1.0)	11 (6.3)
At 3 years (n = 37 325)[b]				
Kicks a ball without support	56 (0.2)	30 (0.1)	12 (1.0)	14 (7.3)
Catches a ball with both hands	898 (2.4)	819 (2.3)	62 (5.1)	17 (8.8)
Holds pencil correctly, like an adult	4230 (11.3)	3915 (10.9)	275 (22.6)	40 (20.7)
Can undo button(s)	3906 (10.5)	3561 (9.9)	283 (23.3)	62 (32.1)

[a] Children with missing data were excluded (n = 4125).
[b] Children with missing data were excluded (n = 1629).

(TABLE 3). The adjusted ORs were 1.04 (95% CI, 0.83-1.30) for other supplements, but no folic acid; 0.82 (95% CI, 0.69-0.97) for folic acid only; and 0.79 (95% CI, 0.68-0.90) for folic acid in combination with other supplements.

We compared maternal report of language development in the age 3 years questionnaire with scores on the Vineland communication domain[16] for 425 children observed clinically as part of a substudy of MoBa.[15] Maternal report and Vineland scores were highly consistent with each other (eFigure).

To assess the specificity of our findings, we analyzed the association between maternal use of dietary supplements and significant delay in gross motor skills at age 3 years. There were 932 children (2.5%) with significant delay in gross motor skills (196 in the no-supplement group, 65 in the no–folic acid supplement group, 163 in the folic acid only group, and 508 in the folic acid plus other supplements group). No association was suggested between maternal intake of folic acid and significant delay in gross motor skills. In a logistic regression analysis adjusted for all potential confounders, we verified that there was no association (eTable 9).

The data on motor development were also used to further characterize the children with severe language delay. A higher proportion of children with severe language delay had not yet attained each of 6 motor skills at 18 months and each of 4 motor skills at age 3 years (TABLE 4). For most children with severe language delay, however, the number of these 10 motor skills that was attained at the specified age were within the normal range (mean [SD], 8.6 [1.3] for no language delay; 7.7 [1.7] for moderate language delay; and 6.5 [2.7] for severe language delay).

COMMENT

Maternal use of supplements containing folic acid within the period from 4 weeks before to 8 weeks after conception was associated with a substantially reduced risk of severe language delay in children at age 3 years. We found no association, however, between maternal use of folic acid supplements and significant delay in gross motor skills at age 3 years. The specificity provides some reassurance that there is not confounding by an unmeasured factor. Such a factor might be expected to relate to both language and motor delay.

To our knowledge, no previous prospective observational study has examined the relation of prenatal folic acid supplements to severe language delay in children. A recent case-control study,[22] based on maternal recall of supplement use several years later, found that use of folic acid supplements in early pregnancy was associated with a reduced risk of autism spectrum disorder. In addition, some prospective studies have reported that folic acid supplementation started before 12 weeks after conception was associated with fewer child cognitive or behavioral difficulties,[23-26] but these studies were too small to examine severe language delay as an outcome. Although it is a rare condition, severe language delay (as defined herein) has profound social and clinical significance. In childhood, it is associated with intellectual disability, neurodevelopmental disorders such as autism, and difficulty achieving literacy.[27] Follow-up studies suggest that, even when there is not an associated intellectual disability, impairment tends to persist into adulthood and is associated with poor literacy.[27]

A major strength of our study was the prospective design, in which pregnant women were followed up from week 17 of pregnancy. Women responded to detailed questions regarding dietary supplement use over 4-week periods, referring to the ingredient lists on the supplement containers. The precision of these data and the large sample size made it possible to differentiate associations with folic acid from associations with other supplements. We achieved this by using 4 mutually ex-

clusive categories of exposure to prenatal supplements, while retaining adequate statistical power to study a rare outcome.

Another strength of our study was that it was conducted in a country, Norway, that does not fortify food with folic acid. In this population, folic acid supplements eclipse dietary sources of folate.[18,28,29] A previous study of a subsample of 2934 randomly drawn pregnancies in the MoBa cohort found a strong correlation between maternal report of folic acid use up to week 17 and plasma folate levels at week 17 of pregnancy.[28]

Because randomized trials that involve withholding usual-care folic acid supplementation are no longer ethical, observational studies must be relied on to examine the implications for child health. Concerns about adverse effects of folic acid supplements on other domains of child[30] and adult[31] health magnify the public health implications. For example, studies have suggested, but not proven, that folic acid supplements may be linked to asthma and atopy in children.[32] The results from this observational study, on the other hand, are strongly suggestive of beneficial effects on child health, but we caution that this study alone is not a sufficient basis for causal inference or policy recommendations.

One central concern is that observational studies of dietary supplements are vulnerable to confounding by behaviors related to health consciousness and socioeconomic circumstances.[33] For several reasons, however, we believe it is unlikely that such confounding explains our main results. First, with respect to prenatal folic acid supplements, prospective observational studies[5] were successful in identifying their relationship to neural tube defects, later validated in randomized controlled trials.[1,2] Perhaps supplement use is more likely to be accurately reported in the health-conscious context of early pregnancy and with a short period of recall required. Second, the magnitude of the association was large, even after adjustment for well-measured potential confounders. Third, the pattern of our results argues against confounding. Exposure to supplements not containing folic acid was not associated with the risk of severe language delay in children. Exposure to folic acid in combination with other supplements (eg, omega-3 fatty acids) showed the same association with severe language delay as exposure to folic acid supplements alone. Moreover, women in 2 of the exposure categories, other supplements, but no folic acid and folic acid only, did not differ appreciably in maternal education levels; however, their children did differ in the risk of language delay. In addition, we found an association for folic acid among women who started these supplements in weeks 5 to 8 after conception. This is not a particularly health-conscious behavior, because folic acid supplements must be taken before week 4 after conception to prevent neural tube defects.

Another central concern is selection bias. To examine the potential for selection bias related to participation in MoBa, we had previously compared 8 exposure-outcome associations in this cohort with the associations in the Medical Birth Registry of Norway and found no evidence of selection bias.[28,34] In this study, we explored the potential for selection bias related to return of the age 3 years questionnaire. Maternal education and folic acid use were associated with the probability of returning the questionnaire, but these associations were found to be independent rather than synergistic, limiting the potential for any resulting selection bias.

In some corollary analyses, the associations we observed were suggestive but not definitive. These results serve to sharpen the challenges for future research. With respect to the relevant period of exposure, the results conform with our a priori hypothesis that folic acid supplements up to week 8 would most likely be associated with a reduced risk of severe language delay. The women who started folic acid supplements after 8 weeks, however, were small in number and not readily comparable with women who started earlier. With respect to a broader spectrum of language delay, we found a statistically robust association with moderate language delay, but it was of a smaller magnitude than for severe language delay. Children with moderate language delay at age 3 years are somewhat heterogeneous, in that a significant number of them will catch up with their peers by age 5 years.[12] One possibility is that a stronger association will be found when the age 5 years MoBa data are available, by excluding children who simply had a different pace of development and had caught up by age 5 years.

In addition, the data available thus far do not permit us to investigate the mechanisms by which folic acid supplements might have a protective effect. The archived biological specimens of the MoBa cohort provide a platform, however, for future research to interrogate genetic, epigenetic, and/or other mechanisms.[35] One intriguing possibility, supported by some animal data,[36] is that folic acid supplements may facilitate reversal or compensation of the epigenetic effects of other early prenatal exposures that disrupt neurodevelopment. Similarly, as suggested by findings on neural tube defects, folic acid may help compensate for genetic variants that confer vulnerability.[37]

In summary, in this large prospective pregnancy cohort in Norway, use of folic acid supplements in the period 4 weeks before to 8 weeks after conception was associated with a reduced risk of the child having severe language delay at age 3 years. If in future research this relationship were shown to be causal, it would have important implications for understanding the biological processes underlying disrupted neurodevelopment, for the prevention of neurodevelopmental disorders, and for policies of folic acid supplementation for women of reproductive age.

Author Contributions: Drs Roth and Magnus had full access to all the data in this study and take responsibility for the integrity of the data and the accuracy of the data analysis. Dr Schjølberg takes responsibility for the integrity of the clinical data.

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