Folic Acid Use and Nonsyndromic Orofacial Clefts in China

A Prospective Cohort Study

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Background: Questions remain about the effectiveness, dose, and timing of folic acid in preventing orofacial clefts. Case-control studies report conflicting results. There have been no cohort studies of orofacial clefts and the use of folic acid without other vitamins.
Methods: In a prospective cohort of 240,244 women enrolled between 1993 and 1995 in 1 northern and 2 southern provinces in China, we examined the risk of nonsyndromic cleft lip with or without cleft palate (CL/P) and cleft palate alone (CP) in relation to maternal use of 400 μg of folic acid without other vitamins.
Results: Daily use of 400 μg of folic acid without other vitamins, started before the last menstrual period (LMP), was associated with reduced risk of CL/P with adjusted rate ratio (aRR) of 0.69 (95% confidence interval = 0.55–0.87). The greatest reduction in risk was observed in the north among daily users who began taking folic acid pills before LMP (aRR = 0.21 [0.10–0.44]); in the south there was marginal reduction in risk (aRR = 0.81 [0.63–1.05]). No evidence of reduced CL/P risk was observed among women who started folic acid pills on or after their LMP. No persuasive evidence for reduction in CP risk was seen with folic acid pill use at any time.
Conclusion: Daily maternal consumption of 400 μg of folic acid without other vitamins, started before mother's LMP, was associated with a reduced risk of CL/P in babies born in a high-prevalence region of China.

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Maternal periconceptional use of multivitamins with or without folic acid has been associated with reduced risk of nonsyndromic orofacial clefts in some, but not all, studies.[1–5] The majority of studies conducted to date have examined the use of multivitamins with folic acid[6–14] or with unspecified folic acid content,[15–19] and 4 studies also reported on dietary folate intake.[10,12,14,19] Five studies have examined maternal use of folic acid without other vitamins.[8,12,20–23] With the exception of the Hungarian cohort[8,9] and Danish case-cohort[13] studies, all have been case-control studies.

The currently available literature[4,5,8,21–23] shows no consistent evidence that periconceptional use of folic acid without other vitamins can reduce the risk of nonsyndromic cleft lip with or without cleft palate (CL/P), or cleft palate alone (CP). Conflicting results might be due to lack of clear timing of folic acid or multivitamin use during the relevant embryologic development period,[7,8,10,15,16,23] lack of information on folic acid dose in multivitamins, or lack of statistical power to detect differences. Three studies that reported reduced risk of CL/P with folic acid or multivitamin use did not observe reduced risk of CP.[12,16,23]

Worldwide, the prevalence of CL/P ranges from 3.4 to 22.9 per 10,000 live births, and the prevalence of CP ranges from 1.3 to 25.3 per 10,000 live births.[24] In China (the Figure), the prevalence of CL/P has been reported to be 11.9 per 10,000 live births in 22 Shanghai hospitals between 1980 and 1989,[25] 14.2 per 10,000 live- or stillborn infants with at least 28 weeks' gestation in the national monitoring system between 1996 and 2005,[26] 14.9 per 10,000 live- or stillbirths with at least 28 weeks' gestation in Shenyang from 2000 to 2007,[23] and 32.7 per 10,000 pregnancies of at least 20 weeks' gestation in 2003–2004 in Shanxi province.[27]

There is an urgent need to identify modifiable etiologic factors to prevent these serious debilitating birth defects.[28]

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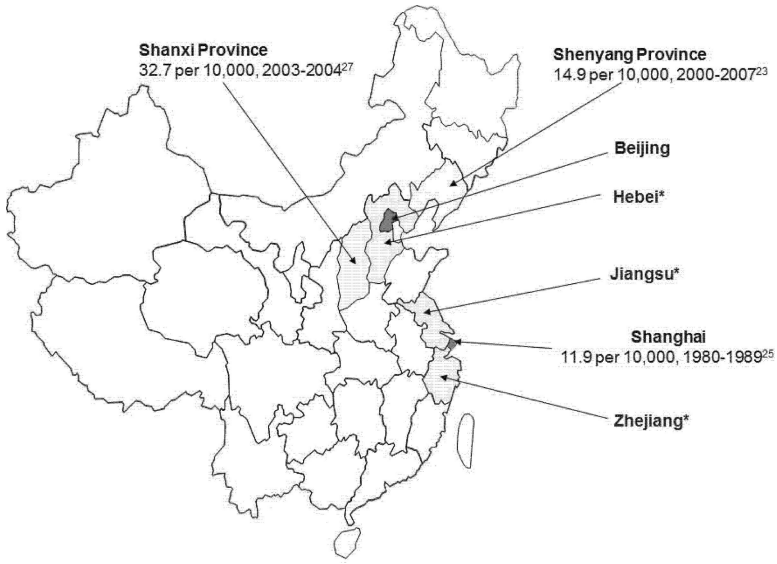

FIGURE. Prevalence of cleft lip with or without cleft palate reported in China. Asterisk indicates provinces that include counties in which the Community Intervention Program operated.

We examined the association of maternal daily consumption of 400 μg of folic acid pills without other vitamins, timing of use (before and during early pregnancy), and compliance of pill use with risk of nonsyndromic CL/P and CP in a large prospective cohort study in China.

METHODS

Study Population

A public health campaign began in October 1993 in China for women to take a folic acid pill without other vitamins to prevent neural tube defects (NTDs). The methods for the campaign have been reported,[29] and are described briefly later in the text. The campaign was conducted in 21 counties in 1 northern (Hebei) and 2 southern (Zhejiang and Jiangsu) provinces of China (the Figure). All pregnant women, as well as all women preparing for marriage, registered with a pregnancy monitoring system that served as the principal record of prenatal care and delivery in all 3 provinces. At registration, women received a physical examination with an interview to obtain demographic information, medical and reproductive history, and family history of birth defects. Women were followed throughout pregnancy until 6 weeks after delivery, or until the termination of pregnancies that did not result in a live birth. The cohort comprised 247,831 pregnant women enrolled from 1 October 1993 through 30 September 1995, who by 31 December 1996 had an informative pregnancy in which the presence of a birth defect in the newborn could be ascertained. The project was approved by the institutional review boards of the U.S. Centers for Disease Control and Prevention and of the Beijing Medical University (now Peking University Health Science Center).

Use of Folic Acid Pills

Beginning in October 1993, all women who registered with the pregnancy monitoring system were asked to purchase pills containing 400 μg of folic acid without other vitamins, and to take 1 pill every day through the end of the first trimester of pregnancy. Women were given 1 bottle containing 31 folic acid pills for each calendar month. At the beginning of each month, village healthcare workers collected the bottles, counted and recorded the number of pills consumed by each woman and the dates women started and stopped taking folic acid pills, as well as the dates of menstrual periods during the month.

We used the classification and pattern of pill taking defined in a paper by Berry et al[29] Women who took any folic acid pill before or during the first trimester of pregnancy were classified as folic acid users for this study. Women who started taking folic acid pills before their last menstrual period (LMP), and who stopped at the end of the first trimester, were considered to be "periconceptional users." Women who started taking folic acid on or after their LMP were considered to be as "late users." Women who started and stopped taking the pills before their LMP comprised the "early discontinuation" group. The "periconceptional use" and "early discontinuation" groups were combined for analyses of "any use before LMP."

We computed the total duration of folic acid pill use, and the compliance of pill use (the percentage of folic acid pills taken compared with the number that could have been taken [≤80% or >80%]). Women who did not agree to take folic acid pills at the time of registration, or who were already in their second trimester of pregnancy at registration (ie, who did not have the opportunity to start taking folic acid pills by the end of their first trimester), were considered "nonusers" of

folic acid and served as the referent group for all comparisons. Women with missing or invalid information for the dates they started and stopped folic acid pill use were excluded from this analysis.

Orofacial Cleft Case Ascertainment

We identified newborns with orofacial clefts through a birth defects surveillance system established in January 1993.[30] This system collected detailed data about infants and fetuses born with external structural birth defects. Included in the surveillance system were live-born infants of at least 20 weeks' gestation who had a birth defect that was diagnosed by 6 weeks of age.[31] We collected information about all pregnancies, including those with gestation of <20 weeks that were electively terminated after the prenatal diagnosis of any birth defect. Three pediatricians unaware of the mothers' folic acid pill use status independently reviewed the reports, photographs, and assigned diagnostic codes. These diagnoses were reviewed and validated by a medical geneticist.[29]

Orofacial clefts in this study are divided into 2 groups, CL/P and CP, considered to be clinically and epidemiologically distinct.[28] Cases of CL/P and CP were further divided by pattern of cleft into 3 groups: (1) "isolated," in which the cleft presented as the only or primary major defect, (2) "multiple," in which the cleft occurred in conjunction with other unrelated external major defects but without a known etiology, or (3) "syndromic," in which the cleft occurred as part of a recognized single gene or chromosomal condition.[32] Characteristics of CL/P and CP included location (midline, bilateral, unilateral, or unknown) and laterality of unilateral orofacial clefts (left, right, or not specified). We also examined cases of cleft lip only (CL) separately from cases of cleft lip with cleft palate (CLP). A medical geneticist classified each case infant without knowledge of maternal folic acid usage.

Based on the widely accepted theory that syndromic cases are pathogenetically and etiologically distinct from other cleft cases,[28] we excluded syndromic cases from this analysis. We also excluded cases diagnosed as Pierre Robin sequence, holoprosencephaly, hemifacial microsomia, and amniotic band sequence, on the basis of pathogenetic and probable etiologic differences. After eliminating these 22 cases, a total of 464 cases remained, 385 with CL/P and 79 with CP.

Statistical Analysis

All women registering with the pregnancy monitoring system on or after 1 October 1993 were asked to take folic acid. We expected most women from this cohort to deliver after 1 July 1994. Our analysis of pregnancy outcome is therefore limited to the period from 1 July 1994 through 31 December 1996. Each woman contributed only one informative pregnancy to the study. For this analysis, we excluded women with missing or invalid information on maternal age, folic acid use, or sex of newborn; multiple births and infants diagnosed with any major or minor external birth defect other than CL/P or CP were also excluded.

We compared the number of women who took folic acid pills and the number who did not in each geographic region according to maternal age (14–19, 20–24, 25–29, or ≥30 years), level of education attained (primary school or less, middle school, high school and beyond, or unknown), occupations of mother and husband or fiancé (farmer, local enterprise, factory, government or business, or unknown), self-reported family history of birth defects (none, any, or unknown), parity (0, 1, ≥2, or unknown), gravidity (1, 2, ≥3, or unknown), gestational weeks at delivery (<28, 28–36, 37–42, or ≥43 weeks), newborn sex (boy or girl), month of birth, and month of mother's LMP (January–March, April–June, July–September, or October–December). We evaluated covariates for potential confounding based on a priori considerations and their observed association with CL/P or CP and folic acid use.

We estimated the prevalence of nonsyndromic CL/P and CP (per 10,000 pregnancies of at least 20 weeks' gestation) by dividing the number of cases of CL/P or CP by the number of pregnancies, multiplied by 10,000. Crude and adjusted rate ratios (RRs and aRRs) for CL/P and CP, and their 95% confidence intervals (CIs), were estimated by categories of folic acid use compared with nonusers using multiple logistic regression modeling. Crude and maternal-age-adjusted RRs[33] are presented in tables because maternal age adjustment resulted in a ≥10% change in estimate compared with crude RR. Adjustment for other covariates did not change these risk estimates. We used the Wald statistic to test for homogeneity of the RR for CL/P and CP in relation to folic acid use by geographic region. Statistical analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Study Participants

Among women who registered with health authorities from 1 October 1993 through 30 September 1995, 240,244 women (31,139 in the north and 209,105 in the south) had an informative pregnancy outcome between 1 July 1994 and 31 December 1996 and fit our inclusion criteria. Fifty-eight percent of women in the north took folic acid pills compared with 52% in the south (Table 1).

Compared with women in the south, women in the north were more likely to have completed middle school, work in farming (herself or husband), and have had no previous pregnancy (Table 1). Women in the south were more likely to deliver before 37 weeks' gestation. Among folic acid pill users in the north, 96% of women reported no previous birth and 93% reported no previous pregnancy; in the south, 92% reported no previous birth and only 51% reported no previous pregnancy.

TABLE 1. Selected Characteristics of Women Enrolled in the Community Intervention Program, by Geographic Region and Folic Acid Pill Use Status, People's Republic of China, 1 October 1993–30 September 1995

	North (n = 31,139)		South (n = 209,105)	
Characteristic	Any Pill Use (n = 18,170) No. (%)[a]	No Pill Use (n = 12,969) No. (%)[a]	Any Pill Use (n = 107,849) No. (%)[a]	No Pill Use (n = 101,256) No. (%)[a]
Age (years)				
14–19	210 (1)	73 (1)	576 (1)	1506 (2)
20–24	14,447 (80)	6312 (49)	86,620 (80)	56,356 (56)
25–29	3007 (17)	3913 (30)	16,248 (15)	26,404 (26)
≥30	506 (3)	2671 (21)	4405 (4)	16,990 (17)
Attained education				
Primary school or less	2328 (13)	1666 (13)	27,002 (25)	33,991 (34)
Middle school	13,619 (75)	9750 (75)	66,710 (62)	54,098 (53)
High school and beyond	1628 (9)	920 (7)	12,196 (11)	9901 (10)
Unknown	595 (3)	633 (5)	1941 (2)	3266 (3)
Occupation				
Farmer	15,396 (85)	11,350 (88)	56,587 (53)	64,948 (64)
Local enterprise	1068 (6)	404 (3)	34,291 (3)	21,195 (21)
Factory	703 (4)	407 (3)	9468 (9)	6978 (7)
Government or business	459 (3)	181 (1)	5726 (5)	5019 (5)
Unknown	544 (3)	627 (5)	1777 (2)	3116 (3)
Occupation of husband/fiancé				
Farmer	15,156 (83)	11,104 (86)	58,765 (55)	62,309 (62)
Local enterprise	1096 (6)	454 (4)	27,755 (26)	19,223 (19)
Factory	871 (5)	534 (4)	9827 (9)	7822 (8)
Government or business	502 (3)	233 (2)	9457 (9)	8582 (9)
Unknown	545 (3)	644 (5)	2045 (2)	3320 (3)
Family history of birth defects[b]				
None reported	17,822 (98)	12,342 (95)	105,961 (98)	97,682 (97)
Any	105 (1)	88 (1)	731 (1)	825 (1)
Unknown	243 (1)	539 (4)	1157 (1)	2749 (2)
Parity				
0	17,411 (96)	8415 (65)	98,954 (92)	74,154 (73)
1	597 (3)	3681 (28)	7937 (7)	24,099 (24)
≥2	24 (0)	158 (1)	496 (1)	1961 (2)
Unknown	138 (1)	715 (6)	462 (0)	1042 (1)
Gravidity				
1	16,975 (93)	7470 (58)	54,612 (51)	42,539 (42)
2	708 (4)	3283 (25)	33,558 (31)	27,489 (27)
≥3	103 (1)	962 (7)	17,964 (17)	27,235 (27)
Unknown	384 (2)	1254 (10)	1715 (2)	3993 (4)
Gestational weeks at delivery				
<28	1192 (7)	467 (4)	5317 (5)	5750 (6)
28–36	5651 (31)	4309 (33)	44,376 (41)	41,753 (41)
37–42	10,802 (59)	7140 (55)	55,777 (52)	49,844 (49)
≥43	231 (1)	168 (1)	1518 (1)	1806 (2)
Unknown	294 (2)	885 (7)	861 (1)	2103 (2)
Sex of newborn				
Boy	9499 (52.3)	6823 (52.6)	55,674 (51.6)	52,996 (52.3)
Girl	8671 (47.7)	6146 (47.4)	52,175 (48.4)	48,260 (47.7)
Newborn's month of birth				
January–March	5290 (29)	3524 (27)	24,197 (22)	23,581 (23)
April–June	2642 (15)	3158 (24)	18,705 (17)	23,901 (24)
July–September	3037 (17)	2652 (20)	26,622 (25)	26,150 (26)
October–December	7201 (40)	3635 (28)	38,325 (36)	27,624 (27)

(*Continued*)

TABLE 1. (*Continued*)

Characteristic	North (n = 31,139) Any Pill Use (n = 18,170) No. (%)[a]	North (n = 31,139) No Pill Use (n = 12,969) No. (%)[a]	South (n = 209,105) Any Pill Use (n = 107,849) No. (%)[a]	South (n = 209,105) No Pill Use (n = 101,256) No. (%)[a]
Mother's month of last menstrual period				
January–March	7227 (40)	3358 (26)	36,979 (34)	26,561 (26)
April–June	5247 (29)	3364 (26)	24,338 (23)	23,793 (24)
July–September	2587 (14)	2946 (23)	18,786 (17)	24,035 (24)
October–December	3109 (17)	2543 (20)	27,745 (26)	25,935 (26)
Unknown	0 (0)	758 (6)	1 (0)	932 (1)

[a]Column percentages do not always sum to 100% due to rounding.
[b]Family history of birth defects includes self-reported maternal or paternal family history of congenital heart or other birth defects.

TABLE 2. Distribution of All Diagnosed Cases of Nonsyndromic Orofacial Cleft by Phenotype

Characteristic	Total No. Cases (%)[a]	North No. Cases (%)[a]	South No. Cases (%)[a]
Nonsyndromic Cleft Lip With or Without Cleft Palate			
Total	385	48	337
Type			
Isolated	364 (95)	46 (96)	318 (94)
Multiple	21 (6)	2 (4)	19 (6)
Location			
Midline	1 (0)	0 (0)	1 (0)
Bilateral	58 (15)	7 (15)	51 (15)
Unilateral	288 (75)	33 (69)	255 (76)
Unknown	38 (10)	8 (17)	30 (9)
Laterality of Unilateral Orofacial Cleft			
Unilateral cases	288	33	255
Left	170 (59)	15 (46)	155 (61)
Right	104 (36)	13 (39)	91 (36)
Not specified	14 (5)	5 (15)	9 (3)
Nonsyndromic Cleft Palate Alone			
Total	79	4	75
Type			
Isolated	77 (97)	4 (100)	73 (97)
Multiple	2 (3)	0 (0)	2 (3)

[a]Column percentages do not always sum to 100% due to rounding.

In both geographic regions (Table 1), compared with nonusers, folic acid users were slightly younger. Users were also more likely to be pregnant for the first time, to report no family history of birth defects, and to deliver between 37 and 42 weeks' gestation.

Orofacial Clefts

Of the 385 cases of CL/P, 61% were boys and 39% were girls, whereas 35% of the 79 CP cases were boys and 65% girls. The majority (95%) of CL/P and CP cases was classified as isolated; 75% of all CL/P cases were unilateral, 59% of unilateral CL/P occurred on the left, and 36% occurred on the right side. Data on location and laterality in children with CP were considered unreliable and are thus not presented. The proportion of CL/P cases with unspecified location or laterality was higher in the north compared with the south (Table 2).

Use of Folic Acid Pills

Overall, 53% (126,019) of women in the study (58% in the north and 52% in the south) used any folic acid pills before or during early pregnancy (Table 3). Among women who used any folic acid pills, the proportion of periconceptional use was 70% in the north and 53% in the south, proportion with late use was 20% in the north and 32% in the south, and the proportion with early discontinuation was 10% in the north and 15% in the south.

Among women who did not take folic acid pills (42% of women in the north and 48% in the south), 3% reported that they decided not to take folic acid, and 67% reported they did not take folic acid because they registered with the pregnancy monitoring system after the first trimester of pregnancy.

Once women began taking folic acid pills, compliance was >90% in all groups in both the north and south (Table 3), with the highest compliance (98%) in the south. The lowest compliance (90%) was in the north in the early discontinuation group.

Orofacial Clefts and Folic Acid Use

The overall prevalence was 16.0 per 10,000 for CL/P and 3.3 per 10,000 for CP (Table 4). Prevalence of CL/P was 15.4 per 10,000 in the north and 16.1 per 10,000 in the south (Table 5).

In the north, prevalence of CL/P was 27.0 per 10,000 among nonusers and 7.2 per 10,000 among folic acid pill users; the prevalence of CL/P in the south was 17.4 per 10,000 among nonusers and 14.9 per 10,000 among folic acid pill users (Table 5).

TABLE 3. Number of Pregnancies and Folic Acid Pill Use Compliance and Duration, by Timing of Pill Use and Geographic Region, Among All Women Who Took Folic Acid Pills

	North (n = 18,170)			South (n = 107,849)		
Folic Acid Pill Use	No. Pregnancies No. (%)	Median Pill-taking Compliance (%)	Median Pill-taking Duration (Months)	No. Pregnancies No. (%)	Median Pill-taking Compliance (%)	Median Pill-taking Duration (Months)
Periconceptional use	12,737 (70)	95.0	6	56,655 (53)	98.6	6
Late use	3640 (20)	96.2	3	34,643 (32)	97.6	3
Early discontinuation	1793 (10)	90.9	5	16,551 (15)	97.5	6

Periconceptional use indicates women who started taking folic acid pills before last menstrual period, and who stopped at the end of the first trimester of pregnancy; Late use, women who started taking folic acid pills on or after last menstrual period; Early discontinuation, women who started and stopped taking folic acid pills before their last menstrual period.

TABLE 4. Number of Cases of Nonsyndromic Cleft Lip With or Without Cleft Palate and Cases of Cleft Palate Alone, Rate per 10,000 Pregnancies, Crude and Age-adjusted Risk Ratios, and 95% Confidence Intervals by Folic Acid Pill Use Characteristics

	Nonsyndromic Cleft Lip With or Without Cleft Palate				Nonsyndromic Cleft Palate Alone			
Folic Acid Pill Use	No. Cases/ No. Pregnancies	Rate per 10,000	Crude RR (95% CI)	Age-adjusted RR (95% CI)	No. Cases/ No. Pregnancies	Rate per 10,000	Crude RR (95% CI)	Age-adjusted RR (95% CI)
Total	385/240,165	16.0			79/239,859	3.3		
None[a]	211/114,185	18.5	1.00	1.00	40/114,014	3.5	1.00	1.00
Any use	174/125,980	13.8	0.75 (0.61–0.91)	0.73 (0.59–0.90)	39/125,845	3.1	0.88 (0.57–1.37)	0.84 (0.53–1.33)
Periconceptional use	93/69,366	13.4	0.73 (0.57–0.93)	0.71 (0.55–0.91)	26/69,299	3.8	1.07 (0.65–1.75)	1.02 (0.61–1.70)
Late use	59/38,274	15.4	0.83 (0.63–1.11)	0.82 (0.61–1.10)	9/38,224	2.4	0.67 (0.33–1.38)	0.65 (0.31–1.34)
Early discontinuation	22/18,340	12.0	0.65 (0.42–1.01)	0.63 (0.41–0.98)	4/18,322	2.2	0.62 (0.22–1.74)	0.59 (0.21–1.68)
Pill use compliance								
≤80%	18/12,069	14.9	0.81 (0.50–1.31)	0.79 (0.49–1.28)	2/12,053	1.7	0.47 (0.11–1.96)	0.45 (0.11–1.87)
>80%	156/113,911	13.7	0.74 (0.60–0.91)	0.72 (0.58–0.90)	37/113,792	3.3	0.93 (0.59–1.45)	0.88 (0.56–1.41)
Any use before LMP	115/87,706	13.1	0.71 (0.57–0.89)	0.69 (0.55–0.87)	30/87,621	3.4	0.98 (0.61–1.57)	0.93 (0.57–1.52)
Pill use compliance								
≤80%	13/9467	13.7	0.74 (0.42–1.30)	0.72 (0.41–1.27)	2/9456	2.1	0.60 (0.15–2.50)	0.59 (0.14–2.46)
>80%	102/78,239	13.0	0.71 (0.56–0.89)	0.68 (0.54–0.87)	28/78,165	3.6	1.02 (0.63–1.66)	1.01 (0.61–1.68)

[a]Reference category.
Periconceptional use, late use, and early discontinuation, as defined in Table 3. Any use before LMP included periconceptional and early-discontinuation users.

Folic acid use before or during pregnancy was not associated with reduced risk of CP (Table 4). Risk was decreased with late use (aRR = 0.65 [95% CI = 0.31–1.34]) and early discontinuation (aRR = 0.59 [0.21–1.68]).

Compared with nonusers (Table 4), folic acid users were at consistently lower risk of delivering a newborn with CL/P; the reduced risk was observed primarily among the periconceptional (aRR = 0.71 [0.55–0.91]) and early discontinuation (0.63 [0.41–0.98]) groups who began folic acid pill use before LMP. This reduced risk was not seen among women who started folic acid later.

The lower risk of CL/P associated with folic acid pill use (Table 5) was observed primarily in the north and not in the south (homogeneity test $P = 0.0004$). In the north, among women with any use of folic acid pills, the aRR for CL/P was 0.24 (0.12–0.46); with any use before LMP, the risk was 0.21 (0.10–0.44). Lower risk was also observed among late users of folic acid pills (0.37 [0.13–1.05]) in the north. In the south, any pill use before LMP was associated with 20% lower risk of CL/P (0.81 [0.63–1.05]) (Table 5).

Cleft lip only (CL) cases accounted for 38% of CL/P overall, with the remainder having cleft lip with cleft palate (CLP). In the north, the proportion of CL/P cases with CL was 29% among nonusers and 44% among women who used pills before LMP. In the south, the proportion of CL/P cases with CL was 39% among nonusers and 41% among women who used pills before LMP (Table 6).

DISCUSSION

Women in northern China who consumed 400 μg of folic acid daily without other vitamins, starting before LMP, had substantially lower risk of nonsyndromic CL/P in their offspring. This reduced risk was observed for both CL and CLP, but not for CP.

TABLE 5. Number of Cases of Nonsyndromic Cleft Lip With or Without Cleft Palate, Rate per 10,000 Pregnancies, Crude and Age-adjusted Risk Ratios, and 95% Confidence Intervals by Folic Acid Pill Use Characteristics and Geographic Region

	Nonsyndromic Cleft Lip With or Without Cleft Palate							
	North				South			
Folic Acid Pill Use	No. Cases/ No. Pregnancies	Rate per 10,000	Crude RR (95% CI)	Age-adjusted RR (95% CI)	No. Cases/ No. Pregnancies	Rate per 10,000	Crude RR (95% CI)	Age-adjusted RR (95% CI)
Total	48/31,135	15.4			337/209,030	16.1		
None[a]	35/12,965	27.0	1.00	1.00	176/101,220	17.4	1.00	1.00
Any use	13/18,170	7.2	0.27 (0.14–0.50)	0.24 (0.12–0.46)	161/107,810	14.9	0.86 (0.69–1.06)	0.84 (0.67–1.05)
Periconceptional use	8/12,737	6.3	0.23 (0.11–0.50)	0.21 (0.10–0.46)	85/56,629	15.0	0.86 (0.67–1.12)	0.84 (0.65–1.10)
Late use	4/3640	11.0	0.41 (0.14–1.14)	0.37 (0.13–1.05)	55/34,634	15.9	0.91 (0.67–1.24)	0.90 (0.66–1.22)
Early discontinuation	1/1793	5.6	0.21 (0.03–1.51)	0.19 (0.03–1.36)	21/16,547	12.7	0.73 (0.46–1.15)	0.71 (0.45–1.13)
Pill use compliance								
≤80%	2/3345	6.0	0.22 (0.05–0.92)	0.20 (0.05–0.84)	16/8724	18.3	1.06 (0.63–1.76)	1.04 (0.62–1.73)
>80%	11/14,825	7.4	0.27 (0.14–0.54)	0.25 (0.12–0.50)	145/99,086	14.6	0.84 (0.68–1.05)	0.83 (0.66–1.04)
Any use before LMP	9/14,530	6.2	0.23 (0.11–0.48)	0.21 (0.10–0.44)	106/73,176	14.5	0.83 (0.65–1.06)	0.81 (0.63–1.05)
Pill use compliance								
≤80%	1/2847	3.5	0.13 (0.02–0.95)	0.11 (0.02–0.82)	12/6620	18.1	1.04 (0.58–1.87)	1.03 (0.57–1.85)
>80%	8/11,683	6.9	0.25 (0.12–0.55)	0.22 (0.10–0.47)	94/66,556	14.1	0.81 (0.63–1.04)	0.80 (0.61–1.03)

[a]Reference category.
Periconceptional use, late use, early discontinuation, and any use before LMP as defined in Tables 3 and 4.

Unlike the higher prevalence of NTD observed in northern compared with southern counties,[29] there was little difference in the overall prevalence of CL/P between the north and south. In the south, the observed prevalence of 16.1 per 10,000 pregnancies was higher than that reported in Shanghai (11.9 per 10,000 live births) in the 1980s,[25] possibly due to the use of different denominators. In contrast, the observed prevalence of 15.4 per 10,000 pregnancies in the north was substantially lower than that reported in Shanxi province in 2003–2004 that used a similar surveillance system and denominator definition, but did not specify exclusion of syndromic cases in its case definition.[27]

In our study, the prevalence of nonsyndromic CL/P among newborns of folic acid users in the north was one-fourth that among newborns of nonusers, whereas there was little difference in the south. One possible explanation for this geographic difference may be that diets in the north were more likely to be folate deficient, and folic acid supplementation compensated for such deficiency. Based on the overall higher socioeconomic status and generally greater availability of fresh vegetables in the southern region, diets in the south were likely more rich in folate than those in the north. This possibility is supported by data from a cross-sectional study showing that significantly higher proportion folate deficiency in the north (40%) compared with the south (6%), based on plasma and red-blood-cell folate concentrations.[34] Another possible explanation for difference by region is that, in the constellation of component causes for CL/P, folic acid deficiency accounts for more cases in the north (suggested by the high prevalence of deficiency among nonusers in the north). We did not have data on possible folate-independent causes of CL/P to test this possibility.

We expected to (but did not) observe decreasing risk of CL/P with increasing duration of folic acid use before LMP. Because duration of pill use in this study is strongly associated with timing of pill use, we were unable to adequately examine duration; we can conclude only that use before LMP is critical. The critical periconceptional period has been established only for neural tube defects. The cells that will eventually form the orofacial region begin to migrate during the first 28 days after conception. Thus, this might be the relevant time period for lip and palate development, not later during gestation as frequently suggested.[4,20] One possible reason for not observing a dose-response relationship is potential misclassification of folic acid pill use duration. It is also possible that increasing duration may be associated with reduced severity of CL/P by decreasing the prevalence of the more severe CLP cases but increasing the prevalence of the less severe CL form. Our results for CL and CLP lend some support for this possibility, but we could not evaluate this further due to limitations in sample size, particularly in the north.

The eTable (http://links.lww.com/EDE/A565) summarizes published studies of folic acid in relation to orofacial clefts. Of 5 studies that presented data on use of folic acid without other vitamins,[8,20–23] 2 Hungarian case-control studies[8,20] reported reduced risk of CL/P with daily use of two 3 mg of folic acid tablets. Two other case-control studies, one in the Netherlands[21] and one in China,[22] examined daily use of 400 μg of folic acid without other vitamins, and neither

TABLE 6. Number of Cases of Nonsyndromic Cleft Lip Only and Cases of Nonsyndromic Cleft Lip With Cleft Palate, Rate per 10,000 Pregnancies, Crude and Age-adjusted Risk Ratios, and 95% Confidence Intervals by Folic Acid Pill Use Characteristics and Geographic Region

	Nonsyndromic Cleft Lip				Nonsyndromic Cleft Lip With Cleft Palate			
Folic Acid Pill Use	No. Cases/ No. Pregnancies	Rate per 10,000	Crude RR (95% CI)	Age-adjusted RR (95% CI)	No. Cases/ No. Pregnancies	Rate per 10,000	Crude RR (95% CI)	Age-adjusted RR (95% CI)
Total	146/239,926	6.09			239/240,019	9.96		
Folic acid use								
None[a]	78/114,052	6.84	1.00	1.00	133/114,107	11.66	1.00	1.00
Any use	68/125,874	5.40	0.79 (0.57–1.09)	0.74 (0.53–1.03)	106/125,912	8.42	0.72 (0.56–0.93)	0.73 (0.56–0.95)
Periconceptional use	34/69,307	4.91	0.72 (0.48–1.07)	0.66 (0.44–1.01)	59/69,332	8.51	0.73 (0.54–0.99)	0.73 (0.53–1.01)
Late use	21/38,236	5.49	0.80 (0.50–1.30)	0.76 (0.47–1.23)	38/38,253	9.93	0.85 (0.59–1.22)	0.85 (0.59–1.23)
Early discontinuation	13/18,331	7.09	1.04 (0.58–1.87)	0.96 (0.53–1.74)	9/18,327	4.91	0.42 (0.21–0.83)	0.42 (0.21–0.83)
Any use before LMP	47/87,638	5.36	0.78 (0.55–1.13)	0.73 (0.50–1.06)	68/87,659	7.76	0.67 (0.50–0.89)	0.67 (0.49–0.90)
				North				
Total	15/31,102	4.82			33/31,120	10.60		
Folic acid use								
None[a]	10/12,940	7.73	1.00	1.00	25/12,955	19.30	1.00	1.00
Any use	5/18,162	2.75	0.36 (0.12–1.04)	0.31 (0.10–0.93)	8/18,165	4.40	0.23 (0.10–0.51)	0.21 (0.09–0.48)
Periconceptional use	3/12,732	2.36	0.31 (0.08–1.11)	0.26 (0.07–0.98)	5/12,734	3.93	0.20 (0.08–0.53)	0.19 (0.07–0.50)
Late use	1/3637	2.75	0.36 (0.05–2.78)	0.31 (0.04–2.45)	3/3639	8.24	0.43 (0.13–1.41)	0.40 (0.12–1.33)
Early discontinuation	1/1793	5.58	0.72 (0.09–5.64)	0.62 (0.08–4.92)	0/1792	0.00	–	–
Any use before LMP	4/14,525	2.75	0.36 (0.11–1.14)	0.31 (0.09–1.01)	5/14,526	3.44	0.18 (0.07–0.47)	0.16 (0.06–0.44)
				South				
Total	131/208,824	6.27			206/208,899	9.86		
Folic acid use								
None[a]	68/101,112	6.73	1.00	1.00	108/101,152	10.68	1.00	1.00
Any use	63/107,712	5.85	0.87 (0.62–1.23)	0.81 (0.57–1.15)	98/107,747	9.10	0.85 (0.65–1.12)	0.86 (0.65–1.15)
Periconceptional use	31/56,575	5.48	0.82 (0.53–1.25)	0.75 (0.49–1.17)	54/56,598	9.54	0.89 (0.64–1.24)	0.91 (0.65–1.27)
Late use	20/34,599	5.78	0.86 (0.52–1.42)	0.81 (0.49–1.34)	35/34,614	10.11	0.95 (0.65–1.39)	0.96 (0.65–1.41)
Early discontinuation	12/16,538	7.26	1.08 (0.58–1.99)	1.00 (0.54–1.86)	9/16,535	5.44	0.51 (0.26–1.01)	0.52 (0.26–1.03)
Any use before LMP	43/73,113	5.88	0.87 (0.60–1.28)	0.81 (0.55–1.20)	63/73,133	8.61	0.81 (0.59–1.10)	0.82 (0.59–1.13)

[a]Reference category.
Periconceptional use, late use, early discontinuation, and any use before LMP as defined in Tables 3 and 4.

observed a reduced risk of CL/P. A case-control study conducted in northern China[23] from 2000 to 2007 reported reduced risk of CL/P with use of folic acid without other vitamins before LMP, but did not specify dose. This study reported no reduction in risk of CL/P with any use of multivitamins or with folic acid use before LMP.[23] Our results in the north show substantially reduced risk of CL/P with 400 μg of folic acid, and thus do not support the need for a higher folic acid dose. The fact that a similar reduction in risk was not observed with the same dose in the south, where we hypothesize women were less likely to suffer folate deficiency, suggests several possibilities. One, a higher dose could be needed to achieve protection. Two, folate-independent factors could play an important role in risk. Three, women in the south may have started folic acid pill use too late during the relevant embryonic development period to achieve protection. Our results are consistent with studies reporting that risk of CL/P is reduced with use of multivitamins containing folic acid, and that use before LMP, regardless of dose, is protective.[6,12,14,16]

One limitation of our study was that folic acid use was not randomized. Women who took folic acid pills may have differed systematically in other factors that could influence the frequency of CL/P. Control for a range of covariates, including maternal education, occupation, family history of birth defects, parity, gravidity, and gestational weeks at delivery, did not change our findings. Because parity and maternal age were correlated, we could not control for their independent effects simultaneously. However, controlling for these measured covariates had a small effect on the risk estimate for CL/P associated with folic acid use.

We did not collect information on maternal smoking, alcohol drinking, use of other vitamin supplements, or dietary folate intake; smoking and drinking among women in China during the study period were uncommon.[35] In the project areas during this study, provision of prenatal vitamins was not part of

prenatal care, and multivitamin supplements were not available for purchase. Although we do not have data on other potential confounders, the population studied was relatively homogeneous. The general lifestyle among women in rural China, including local housing, access to health care, and healthcare seeking behavior, tended to be uniform. The main difference between folic acid pill users and nonusers was that most nonusers registered for prenatal care at their first prenatal visit near the end of the first trimester of pregnancy.[36]

Another limitation is that only external birth defects were included in the surveillance system. CP could easily be missed when looking for external defects, and under-ascertainment of infants with CP likely affected our ability to evaluate the relationship between CP and folic acid use. In addition, without ascertainment of internal birth defects, we do not know how many infants classified as "isolated" actually had multiple defects. A recent U.S. study[37] found that approximately 13% of infants with multiple congenital anomalies had CL/P and 21% had CP. However, we would expect those figures to be lower in our study because information on internal defects was not collected. Finally, the designation of "multiple" or "syndrome" was likely an underestimate, as this was based on photographs alone, with limited ability to evaluate clinical presentation and phenotype.

The strengths of this study include the population-based nature of the study, nearly complete ascertainment of major external birth defects among the newborn of large numbers of women whose pregnancies lasted at least 20 weeks, the use of folic acid pills with known dose and without other vitamins, and the prospective monthly recording of folic acid use before the pregnancy outcome was ascertained. The prospective surveillance system for birth defects was established before our evaluation began, with birth defects diagnoses based on reviews of reports and photographs by several clinicians.

We conclude that daily maternal consumption of 400 μg of folic acid without other vitamins before the mother's LMP can reduce the risk of CL/P in newborns in a high prevalence region. The risk of CL/P was apparently reduced by 20%–80%. These results add to the available evidence that the risk of CL/P can be modified by periconceptional folic acid use. The preventive effect of folic acid use before and during early pregnancy on other non-NTD birth defects, including cleft palate alone, needs further exploration.

The United States Public Health Service recommends that all women who could become pregnant take 400 μg of folic acid daily to reduce the risk of NTDs.[38] Other countries have adopted this recommendation[39]; in 2009, the Chinese Ministry of Health decided to provide free folic acid supplements to rural women considering pregnancy throughout China to prevent NTDs. Our results suggest that the implementation of such recommendations might also help reduce the risk of CL/P.

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