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An investigation of the variation of level of lesion of spina bifida in all births in the presence and absence of maternal folic acid supplementation in China, 1993-1996

By

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Masters of Science in Public Health

Epidemiology

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An investigation of the variation of level of lesion of spina bifida in all births in the presence and absence of maternal folic acid supplementation in China, 1993-1996

By

Calvin Patimeteeporn

B.A., Vanderbilt University 2013

Faculty Thesis Advisor: Dr. Michael Goodman, MD MPH

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Abstract

An investigation of the variation of level of lesion of spina bifida in all births in the presence and absence of maternal folic acid supplementation in China, 1993-1996
By Calvin Patimeteeporn

Introduction: Folic acid (FA) supplementation has been conclusively shown to prevent neural tube defects (NTDs), including spina bifida. However, the potential impact of FA supplementation on the severity of the spina bifida, as estimated by highest level of the lesion, requires more research.
Methods: Birth outcomes of a cohort of Chinese women who were enrolled into a perinatal health care system between 1993 and 1996 in two regions in China, were evaluated to assess the relation between the location of the spina bifida lesion and the any use of FA supplement prior to the end of the woman's first trimester.
Results: 540,000 women were included in the final analysis and of those women, 242 women had a spina bifida affected pregnancy with level of lesion information available. There was an overall decrease in prevalence of spina bifida in the study population by FA supplement exposure, though the effect appeared to vary by region. Spina bifida cases residing in the southern region were twice as likely to have high level of lesion, as were spina bifida cases in the northern region. Using polytomous logistic regression models stratified by child's sex and region and adjusted for maternal age, a statistically significant difference in prevalence of high level of lesion spina bifida was observed in the northern but not in the southern region.
Conclusions: The impact of FA supplementation on the change in prevalence of high level of lesion spina bifida appears to vary by region, possibly dependent on the baseline prevalence of NTDs existing in the population prior to the introduction of FA. This observation may be explained by genetic or environmental factors or a combination of both. More research is needed to clarify the roles FA and other factors in spina bifida presentation.

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Background/Literature Review

Neural tube defects (NTDs) affect more than 300,000 newborns globally each year, making these the most common congenital anomalies worldwide (1). NTDs are severe abnormalities of the central nervous system and originate from the incomplete closure/fusion of the neural tube in the developmental period of the human embryo (2). NTDs may have a variety of manifestations, and depending on the nature of lesion and location are classified as spina bifida, anencephaly, or encephalocele as well as other rare types that most commonly occur in high prevalence areas (3). Among these NTDs, spina bifida is the most common. This type of defect occurs when there is a failure of fusion of the caudal portion of the neural tube and concomitant schisis of the outside of the vertebrae (3). Even though spina bifida has a relatively high survival rate in high-resource countries, it is usually associated with serious lifelong disability, most notably paralysis (4).

The Centers for Disease Control and Prevention (CDC) reports that in the United States, approximately 50% of babies born with NTDs are diagnosed with spina bifida. This makes spina bifida the most common type of the NTDs in the United States. The severity of spina bifida depends on a variety of factors, one of which is the location of the lesion along the spine. Differing locations of the malformations along the spine is associated damage to nerves vital to muscle function and sensation both at the location of lesion and any nerves below. Thus, the higher the location of the lesion, the greater chance for more significant nerve damage (5).

While, the association between higher risk of adverse complications and location of lesion of spina bifida are well known, the patterns of location of lesion in populations

and possible casual factors have not been studied in depth. In 1988, Hall and colleagues noted trends in location of lesion among East-Indian Sikh populations and non-Sikh populations in British Columba (6). In this study, higher locations of spina bifida was more prevalent in Sikh populations, providing evidence that there may be some alternate factor influencing the location of the lesion along the spine in certain populations. In addition, in 1991, a EUROCAT work group found that there was a much higher reported proportion of higher locations of spina bifida (37.4%) in the United Kingdom when compared to continental Europe (14.4%) (7). In addition, there have been many studies on spina bifida type concordance among siblings. Many studies have shown that the type concordance among siblings is significantly high (8, 9). However, there have been conflicting studies that did not observe this same frequency of concordance among siblings (10). Thus, much more research in examining possible genetic or hereditary factors contributing to level of lesion is needed.

A study showed evidence of the effect of a genetic component, specifically the gene coding for the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) in the presentation of spina bifida among pregnancies to Hispanic women (11). In infants with homozygosity for the 677TT variant of the gene, a statistically significant association with location of lesion, particularly higher lesions was found.

There is convincing evidence that adequate maternal (FA) intake reduces the risk of NTDs, including spina bifida (12-14). This evidence has prompted a recommendation of routine FA supplementation for women of childbearing age. The USPHS recommends that all women of childbearing age take 400 micrograms of FA daily (15). As a result, the US there was a 31% drop in spina bifida prevalences and the overall number of NTD-

affected pregnancies declined from 4,000 in 1995- 1996 and to 3,000 in 1999-2000 (16). Despite these efforts, even in the United States where almost all women are provided with FA-fortified foods and have access to supplements, approximately 1,500 babies are born with spina bifida each year (17).

In addition to the reports on the effectiveness of FA supplementation in the US, there has been much evidence supporting FA interventions for decreasing NTD prevalences in other countries. For example, the number of NTD-affected births dropped by 44% after the introduction of FA fortification in wheat flour for bread in Chile (18).

In the past, China also experienced high prevalences of NTDs, which resulted in a public health campaign of maternal use of folic acid to prevent NTDs (14). As part of this effort, women planning marriage in two regions of the country, Hebei province in the North and Zhejiang and Jiangsu provinces in the South were asked to take supplements containing only 400 micrograms of FA and were given information about the benefits of using folic acid to prevent NTDs (14). Information about all pregnancies in both regions were obtained from a pregnancy monitoring system, which was the main source for medical record keeping for pregnant, married, or preparing to be married women. All women, regardless of their FA intake, were followed until the outcome of their pregnancy was known and recorded. A total of 247,831 pregnant women were included in the evaluation of the use of folic acid to prevent NTDs. The evaluation took place from October 1993 to December 1996 and found a statistically significant decrease in NTD birth prevalence in both populations demonstrating that 400 micrograms of folic acid without other vitamins prevented NTDs; however the magnitude of the reduction was different in the two regions: 79% in the north and 16% in the south.

Despite the clear success of the intervention in both regions a number of children were born with NTDs and the effect of FA supplementation appeared to differ depending on the study population. In addition, it was noted that the distributions of NTD location and severity may have been affected by FA intake, as reported in one study in 2008 (21). An examination of the effects of folic acid fortification of grains in Canada revealed not only an overall drop in total NTDs, but a significant drop in the high location of the lesion of spina bifida in infants from pre-fortification to after fortification. The study found that the introduction of folic acid almost completely erased the previous regional differences of NTD prevalences in various provinces across Canada. Using this as a launching point, further research is needed to determine if folic acid is a factor in influencing the location of the spina bifida.

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Introduction

Neural tube defects (NTDs) affect more than 300,000 newborns globally each year, making these one of the most common congenital anomalies worldwide(1). NTDs are severe abnormalities of the central nervous system and originate from the incomplete closure/fusion of the neural tube in the developmental period of the human embryo (2). NTDs may have a variety of manifestations, and depending on the nature of lesion and location are classified as spina bifida, anencephaly, or encephalocele as well as other rare types that most commonly occur in high prevalence areas (3). Among these NTDs, spina bifida is the most common. This type of defect occurs when there is a failure of fusion of the caudal portion of the neural tube and concomitant schisis of the outside of the vertebrae (3). Even though spina bifida has a relatively high survival rate in high-resource countries, it is usually associated with serious lifelong disability, most notably paralysis (4).

According to the US Centers for Disease Control and Prevention (CDC), approximately 50% of babies born with NTDs are diagnosed with spina bifida. This makes spina bifida the most common type of NTDs in the United States. Differing levels of the malformations along the spine damage nerves both at the level of lesion and any nerves below that are vital to muscle function and sensation. Thus, the higher the level of the lesion, the greater the severity of disability (5).

There is convincing evidence that adequate maternal (FA) intake reduces the risk of spina bifida and other NTDs (6, 7). This evidence has prompted a recommendation of routine FA supplementation for women of childbearing age (8). In 1992, USPHS recommended that all women of childbearing age should take 400 micrograms of FA

daily (8). In addition, a number of countries instituted folic acid fortification of cereal grains, such as the United States, and results have shown that these interventions have produced a reduction in neural tube defects overall (9). As a result, in the US there was a 31% drop in spina bifida prevalences and the overall number of NTD-affected pregnancies declined from 4,000 in 1995-1996 and to 3,000 in 1999-2000 (10). Despite these efforts, even in the United States where almost all women are provided with FA-fortified foods and have access to supplements, approximately 1,500 babies are born with spina bifida each year (11).

In the past, China also experienced high prevalences of NTDs, which stimulated a public health campaign (12). As part of this effort, all women in two regions of the country, the Hebei province in the north and the Zhejiang and Jiangsu provinces in the south were asked to purchase and take FA supplements and were given information about the benefits of using the vitamin. Following the intervention, which took place from October 1993 to December 1996 there was a statistically significant decrease in NTD prevalence in both populations; however the magnitude of the reduction was different in the two regions – 79% in the north and 16% in the south – demonstrating that 400 micrograms of folic acid alone prevents NTDs.

In addition, it was noted that the distributions of NTD location and severity may have been affected by FA intake, as reported in one study in 2008 (13). An examination of the effects of folic acid fortification of grains in Canada revealed not only an overall drop in total NTDs, but a significant drop of higher level spina bifida in infants from pre-fortification to after fortification. It saw that the introduction of folic acid almost

completely erased the previous regional differences of NTD prevalences in various provinces across Canada.

The purpose of the current study is to address this issue in another population by evaluating the severity of spina bifida that occurred among offspring of women who received FA relative to those who had no supplementation.

Methods

The participants were pregnant, married, or preparing to be married women who had registered with a pregnancy-monitoring system within the two regions in China. The database included all women who had an informative birth outcome between January 1, 1993 and the end of December 1996.

In China, all women were mandated to attend a premarital examination, which includes a physical examination in addition to laboratory tests. From October 1, 1993 through September 30, 1995, all women who attended one of these examinations were recommended to purchase and take 400 micrograms of FA once a day until the end of the first trimester of pregnancy. Village health care providers were asked to keep a monthly record of the FA supplement regimen for these women.

The extent of compliance with recommendations was used to measure exposure to FA s among the cohort members. For the current analysis, all participants were characterized as either any FA pill users or non-users. Women included in the first group would have had taken FA pills at some point, regardless of the total dose, before the end of the first trimester of pregnancy.

NTD cases were identified via a Birth Defects Surveillance System, which was established in 1992 (14). The system recorded detailed information on infants and fetuses with external birth defects. In addition, a standard set of photographs were taken of all stillborn infants and all newborns with birth defects. Three pediatricians, who were unaware of the mother's pill-taking status, independently assessed the photographs and the information provided by the surveillance system. A clinical geneticist was then consulted to validate the diagnoses. NTDs were characterized as anencephaly, spina bifida, iniencephaly, craniorachsichisis, or encephalocele. Spina bifida cases were further sub-classified according to the location of the lesion on the spine based on photos. In the current analysis, location of the lesions was classified as high level (cervical and thoracic) or low level (lumbar and sacral).

A total of 666,925 women registered with health authorities during the study period, however after excluding those with invalid dates of birth and unknown birth outcomes, the study population included 629,484 women. The cohort was further reduced after excluding women whose FA supplementation exposure was unknown. This would exclude women with an "unknown" or missing value as well as women who registered after September 30, 1995 when pill-taking status was no longer recorded. The pill-taking cohort included 540,000 women and 580 reported cases of NTDs. Prevalence estimates for NTDs and spina bifida were calculated and compared across the two intervention groups by study region and by sex. In addition prevalence of high vs. low level of spina was assessed. All prevalence estimates were expressed per 10,000 pregnancies of $\geq$ 20 week gestation. Fischer's Exact test was used to assess any significance between regional prevalence.

To see the change of prevalence of types of spina bifida among all births, the association between level of the lesion in spina bifida cases and FA exposure among the entire cohort was examined using a polytomous logistic regression model which controlled for maternal age (continuous) and was stratified by both region and child's sex. To perform this analysis, the outcome variable was a three level term: high spina bifida, low spina bifida, no spina bifida. Maternal age was adjusted for, as it was the only covariate to be significantly associated with both exposure and outcome. The models were examined for two way interactions and for collinearity. The results of logistic regression analyses were expressed as adjusted odds ratios (ORs) and the corresponding 95% confidence intervals (CIs). All statistical analysis was performed using SAS software (version 9.2; Statistical Institute, Cary, NC, USA).

Results

The study population included 629,484 women; 92,606 in the northern region and 536,878 in the southern region (Table 1). Participants in the two geographical regions differed with respect to maternal occupation and education as well as parity.

Of the 540,000 women and 580 reported cases of NTDs that had a valid exposure variable, 242 of the NTD cases were spina bifida (Table 2). Prevalence of spina bifida was also calculated for this new cohort (Table 3). Prevalence of spina bifida is seen to be much higher in females than in males as well as a much higher overall prevalence in the northern region. The level of lesion of spina bifida cases by region, sex, and exposure status is presented in Tables 4. The prevalence estimates for both high and low spina bifida lesions were clearly lower in the FA supplementation group than among pill non-

takers in the northern region. By contrast the corresponding differences in the southern region were less pronounced. Among male offspring in the southern region the prevalence of high spina bifida was slightly higher in the FA exposed group than in the reference category. It can be seen that there was only a statistically significant association between the two factors in the northern study population, which can be seen in Table 4.

Table 5 and Table 6 present the results of the analyses examining the association between FA and high and low spina bifida among the birth cohort. As predicted in the Fischer's Exact test, FA has a significant impact on the development of high spina bifida in the northern region only (Overall north OR = 0.30; 95% CI: 0.14, 0.66). This significant effect of FA disappears in the southern region. Maternal age was also shown to be a significant risk factor for high spina bifida in northern females (OR = 1.04, 95% CI: 1.01, 1.07). In contrast, when examining the association between FA and low spina bifida, none of the odds ratios were significant.

Discussion

The results of this analysis indicate that FA supplementation has a significant impact on the prevalence of high spina bifida, but this relationship is only seen in the northern region of the study population. FA supplementation was seen to have a statistically significant protective effect on the prevalence of high spina bifida among all births in this region only. This association was reflected in both the Fischer's Exact test and the polytomous logistic regression models for the northern region. These tests were also stratified by sex, however, outside of a higher prevalence of high spina bifida in

females than males in the unexposed group, the regression models revealed no overall significant differences in the effect of FA on each sex, except in northern females.

The literature on the association between FA supplementation and spina bifida level is sparse. To our knowledge only one previous study observed a significant decrease in high spina bifida after fortification of cereal products with FA (13). In that study, conducted in Canada, De Wals and colleagues observed that after FA fortification, regional differences of spina bifida prevalences and heterogeneity were almost completely eliminated. These data suggested that folate insufficiency was the primary factor determining spina bifida presentations in different parts of the country. In addition, the fortification also appeared to have greater effect on high spina bifida, but this effect was only seen in regions with baseline elevated birth prevalence of NTDs (13).

The association between high spina bifida and geographic region in this population has a number of possible explanations. It is possible that persons living in the north are more susceptible to FA insufficiency. In addition, since the northern region had a much higher prevalence of NTDs, the impact of FA was much easier to see, as opposed to the southern region. One previous study reported that the gene encoding 5,10-methylenetetrahydrofolate reductase (MTHFR), might affect presentation of spina bifida among pregnancies to Hispanic women. (15). The homozygous 677TT variant of the gene, which is the less active variant, was associated with statistically significant increase in the proportion of high spina bifida. This is particularly relevant as Crider and colleagues assessed the prevalence of genotype variants in Northern China, and found that 35.1% of the sampled population displayed the homozygous 677TT variant (16).

This could possibly explain the high spina bifida prevalence observed in the northern study population.

It is also possible that nutritional status of women in the North may be different from that in South. As seen in the analysis, the prevalences of NTDs in the northern study population differed greatly from the southern region (12). Thus, suggesting that baseline nutritional status may have differed in the two regionally distinct populations, which could explain the difference in effect of the folic acid intervention. As hypothesized in the 2008 Canadian study, the reduction in spina bifida birth prevalence may vary by the baseline prevalences of NTDs prior to intervention (13).

In general, location of spina bifida lesions has been shown to vary in differing populations. For example a 1988 Canadian study observed that high spina bifida lesion were particularly common among East-Indian Sikhs compared to non-Sikh populations of British Columba (17). Higher proportions of high spina bifida was also observed in the United Kingdom and Ireland relative to continental Europe (18). In addition, several studies have shown concordance of level of lesion of spina bifida among siblings (19).

Maternal age was shown to be a significant factor for one of the stratum of region and child's sex analyzed, and showed to have a slightly positive effect. In addition, while most of the estimates for maternal age's effect on high and low spina bifida were not significant, a number of them had an OR greater than 1. This could be possibly explained due to the nature of how the distribution of folic acid information was performed. Since women in premarital counseling sessions were told about folic acid and its potential benefits, older women who were becoming pregnant again would not have been told of these effects until much later until after they had become pregnant and would be

classified as unexposed. Thus, maternal age could be seen as a factor for developing spina bifida due to lack of knowledge of FA.

An important limitation of the current analysis is the possible misclassification of exposure. FA supplementation was assessed during the first trimester of pregnancy and thus the exposed group may have included women who did not take supplements on a regular basis, who stopped before their LMP or who started after their LMP. However, further examination in the pill taking status of these individuals showed high compliance (14). Another limitation is that despite elevated prevalences of NTDs, the number of cases of spina bifida was low, and further division according to the level of lesion further reduced statistical power particularly in the southern region where prevalences were approximately 10-fold lower than in the northern region. In addition, since the data collected on level of spina bifida lesion was assessed using anatomical references in photographs only, there is also the potential for outcome misclassification.

These limitations notwithstanding, the current study makes an important contribution to the literature on the association between FA exposure and NTD risk. The data were obtained from large populations that had information for all births within the study provinces limiting the opportunity for selection bias.

In summary, the results of our analyses have further expanded our understanding between FA and level of lesion of spina bifida, however leaves much room for future steps. In the region known for elevated NTD risk the inverse association between FA and overall occurrence of spina bifida was primarily attributable to a decrease in high-level lesions. By contrast, in the region with lower NTD risk the result of FA supplementation, although still detectable overall, did not appear to differ in terms of spina bifida location,

confirming with previous literature on the effects of FA on high and low spina bifida prevalence. These findings indicate that the role of FA in NTD pathophysiology maybe complex, multifactorial, and subject to substantial modification by genetic and environmental factors. The interactions between FA and these factors require further study.

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Tables

Table 1: Selected characteristics of women enrolled in the public health campaign and gave birth. Data presented by geographic region, People's Republic of China, 1993 to 1996

Characteristics	North		South		Total	
of Study Participants	**N**	**%**	**N**	**%**	**N**	**%**
Age (years)						
14 to 19	106	0.1%	1,087	0.2%	1,193	0.2%
20 to 24	30,766	33.2%	202,737	37.8%	233,503	37.1%
25 to 29	44,365	47.9%	255,999	47.7%	300,364	47.7%
30 or older	16,193	17.5%	72,940	13.6%	89,133	14.2%
Missing	1,176	1.3%	4,115	0.8%	5,291	0.8%
Total	92,606		536,878		629,484	
Gestational age (weeks)						
Less than 28	833	0.9%	1,940	0.4%	2,773	0.4%
28-36	3,380	3.6%	17,958	3.3%	21,338	3.4%
37-42	75,285	81.3%	466,246	86.8%	541,531	86.0%
Over 42	13,088	14.1%	50,666	9.4%	63,754	10.1%
Missing	20	0.0%	68	0.0%	88	0.0%
Total	92,606		536,878		629,484	
Education						
Primary School or less	10,883	11.8%	135,824	25.3%	146,707	23.3%
Middle School	56,717	61.2%	302,718	56.4%	359,435	57.1%
High School and Beyond	22,501	24.3%	7,8604	14.6%	101,105	16.1%
Missing	2,505	2.7%	19,732	3.7%	22,237	3.5%
Total	92,606		536,878		629,484	
Occupation						
Farmer	60,376	65.2%	280,672	52.3%	341,048	54.2%
Local Enterprise	3,309	3.6%	126,560	23.6%	129,869	20.6%
Worker/Laborer	16,366	17.7%	67,951	12.7%	84,317	13.4%
Government or business	8,726	9.4%	28,856	5.4%	37,582	6.0%
Missing	3,829	4.1%	32,839	6.1%	36,668	5.9%
Total	92,606		536,878		629,484	
Sex of child						
Male	48,818	52.7%	280,046	52.2%	328,864	52.2%
Female	43,377	46.8%	255,346	47.6%	298,723	47.5%
Missing	411	0.4%	1,486	0.3%	1,897	0.3%
Total	92,606		536,878		629,484	

Table 1: Selected characteristics of women enrolled in the public health campaign by geographic region, People's Republic of China, 1993 to 1996 *(continued)*

Characteristics of Study Participants	North N	North %	South N	South %	Total N	Total %
Family History of BD						
Any	446	0.5%	1,504	0.3%	1,950	0.3%
None Reported	89,698	96.9%	517,170	96.3%	606,868	96.4%
Unknown	2,462	2.7%	18,204	3.3%	20,666	3.3%
Total	92,606		536,878		629,484	
Gravidity						
1	63,627	68.7%	267,164	49.8%	330,791	52.5%
2	15,872	17.1%	147,426	27.5%	163,298	25.9%
3 or more	12,147	13.1%	118,163	22.0%	130,310	20.7%
Missing	960	1.0%	4,125	0.8%	5,085	0.8%
Total	92,606		536,878		629,484	
Parity						
0	6,749	7.3%	172,825	32.2%	179,574	28.5%
1	12,646	13.7%	75,313	14.0%	87,959	14.0%
2 or more	700	0.8%	6,174	1.1%	6,874	1.1%
Missing	72,511	78.3%	282,566	52.7%	355,077	56.4%
Total	92,606		536,878		629,484	

Table 2: Frequency of selected NTDs in the births of women enrolled in the public health campaign by geographic region, People's Republic of China, 1993 to 1996

Year and type of NTD	North (total N=282)		South (total N=413)		Total (total N=695)	
	N	%	N	%	N	%
1993						
Anencephaly	40	36%	61	52%	101	44%
Spina Bifida	60	54%	35	30%	95	41%
Encephalocele	11	10%	22	19%	33	14%
Total	111		118		229	
1994						
Anencephaly	29	38%	41	49%	70	43%
Spina Bifida	39	51%	25	30%	64	40%
Encephalocele	9	12%	18	21%	27	17%
Total	77		84		161	
1995						
Anencephaly	20	40%	46	50%	66	46%
Spina Bifida	25	50%	32	35%	57	40%
Encephalocele	5	10%	14	15%	19	13%
Total	50		92		142	
1996						
Anencephaly	2	17%	16	44%	18	38%
Spina Bifida	9	75%	17	47%	26	54%
Encephalocele	1	8%	3	8%	4	8%
Total	12		36		48	
Total						
Anencephaly	91	36%	164	50%	255	44%
Spina Bifida	133	53%	109	33%	242	42%
Encephalocele	26	10%	57	17%	83	14%
Total	250		330		580	

Note: Any birth with an invalid exposure value or unknown or ambiguous child's sex were excluded

Table 3: Prevalence (P) per 10,000 pregnancies of ≥ 20 week gestation and frequency of spina bifida in the northern region of women enrolled in the public health campaign by child's sex and folic acid (FA) exposure, People's Republic of China, 1993 to 1996

North	**Male (n=41,361)**				**Female (n=36,716)**			
Year	**FA (n=11,310)**		**No FA (n=30,051)**		**FA (n=10,226)**		**No FA (n=26,490)**	
	P (95% CI)	**(N)**	**P (95% CI)**	**(N)**	**P (95% CI)**	**(N)**	**P (95% CI)**	**(N)**
1993	N/A	N/A	20.4 (11.7-27.0)	27	N/A	N/A	28.9 (19.9-40.6)	33
1994	8.6 (1.0-30.9)	2	20.1 (11.7-32.1)	17	4.8 (0.1-26.5)	1	25.4 (15.4-39.7)	19
1995	10.7 (3.9-23.4)	6	10.7 (3.9-23.4)	6	7.9 (2.1-20.1)	4	17.5 (8.0-33.1)	9
1996	3.0 (0.04-16.4)	1	3.6 (0.1-20.1)	1	13.2 (3.6-33.6)	4	12.2 (2.5-35.4)	3
Total	8.0 (3.6-15.1)	9	17.0 (12.6-22.3)	51	8.8 (4.0-16.7)	9	24.2 (18.6-30.8)	64
South	**Male (n=240,536)**				**Female (n=221,387)**			
Year	**FA (n=**63,886**)**		**No FA (n=**176,650**)**		**FA (n=**59,777**)**		**No FA (n=**161,610**)**	
	P (95% CI)	**(N)**	**P (95% CI)**	**N**	**P (95% CI)**	**(N)**	**P (95% CI)**	**N**
1993	N/A	N/A	1.2 (0.5-2.4)	8	N/A	N/A	4.3 (2.9-6.3)	27
1994	0.6 (0.04-3.1)	1	1.3 (0.5-2.6)	7	1.2 (0.1-4.5)	2	3.0 (1.6-5.0)	15
1995	1.3 (0.4-3.4)	4	2.2 (1.0-4.3)	9	2.5 (1.0-5.1)	7	3.3 (1.7-5.8)	12
1996	0.6 (0.04-3.4)	1	1.4 (0.1-5.1)	2	5.1 (2.2-10.1)	8	4.5 (1.7-9.8)	6
Total	0.9 (0.3-2.0)	6	1.5 (1.0-2.2)	26	2.8 (1.6-4.6)	17	3.7 (2.9-4.8)	60

Note: There are no values for FA groups in 1993 as the earliest pill taking began in late 1993 and the first births for these groups occurred in 1994

Table 4: Prevalence (P) per 10,000 pregnancies of ≥ 20 week gestation of high and low spina bifida in the northern and southern region of women enrolled in the public health campaign by child's sex and folic acid (FA) exposure, People's Republic of China, 1993 to 1996

North	**Male (n=41,361)**					**Female (n=36,716)**					**Total (n=78,077)**				
Level of Lesion	**FA (n=11,310)**		**No FA (n=30,051)**		**P-value**	**FA (n=10,226)**		**No FA (n=26,490)**		**P-value**	**FA(n=21,536)**		**No FA (n=56,541)**		**P-value**
	P (95% CI)	**N**	**P (95% CI)**	**N**		**P (95% CI)**	**N**	**P (95% CI)**	**N**		**P (95% CI)**	**N**	**P (95% CI)**	**N**	
High	2.7 (0.5-7.7)	3	9.0 (5.9-13.1)	27	0.04	3.9 (1.1-10.0)	4	13.2 (9.2-18.4)	35	0.01	3.3 (1.3-6.7)	7	11.0 (8.4-14.1)	62	<0.01
Low	5.3 (1.9-11.5)	6	8.0 (5.1-11.9)	24	0.50	3.9 (1.1-10.0)	4	8.7 (5.5-13.0)	23	0.18	4.6 (2.2-8.6)	10	8.3 (6.1-11.0)	47	0.11
Total	8.0 (3.6-15.1)	9	17.0 (12.6-22.3)	51	0.03	7.8 (3.4-15.4)	8	21.9 (16.6-28.3)	58	0.003	7.9 (4.6-12.7)	17	19.3 (15.9-23.3)	109	<0.01
South	**Male (n=240,536)**					**Female (n=221,387)**					**Total (n=461,923)**				
Level of Lesion	**FA (n=63,886)**		**No FA (n=176,650)**		**P-value**	**FA (n=59,777)**		**No FA (n=161,610)**		**P-value**	**FA(n=123,663)**		**No FA (n=338,260)**		**P-value**
	P (95% CI)	**N**	**P (95% CI)**	**N**		**P (95% CI)**	**N**	**P (95% CI)**	**N**		**P (95% CI)**	**N**	**P (95% CI)**	**N**	
High	0.5 (0.1-1.3)	3	0.3 (0.2-0.7)	6	0.89	1.0 (0.3-2.2)	6	1.4 (0.9-2.1)	23	0.59	0.7 (0.4-1.4)	9	0.9 (0.6-1.2)	29	0.83
Low	0.5 (0.1-1.3)	3	1.0 (0.6-1.6)	18	0.30	1.8 (0.9-3.3)	11	1.9 (1.3-2.7)	30	0.99	1.1 (0.7-1.9)	14	1.4 (1.1-1.9)	48	0.56
Total	0.9 (0.3-2.0)	6	1.4 (0.9-2.0)	24	0.56	2.8 (1.6-4.6)	17	3.3 (2.5-4.3)	53	0.72	1.9 (1.2-2.8)	23	2.3 (1.8-2.8)	77	0.47

Table 5: Polytomous logistic regression analysis evaluating factors associated with high spina bifida among all of women enrolled in the public health campaign by child's sex and folic acid (FA) exposure, People's Republic of China, 1993 to 1996

North	**Male**		**Female**		**Total**	
Factor	**Odds Ratios**	**95% Confidence Intervals**	**Odds Ratios**	**95% Confidence Intervals**	**Odds Ratios**	**95% Confidence Intervals**
Folic Acid Supplementation						
None	*(ref.)*		*(ref.)*		*(ref.)*	
Any	0.28*	(0.09, 0.93)	0.31*	(0.11, 0.87)	0.30*	(0.14, 0.66)
Maternal age (continuous)	0.96	(0.85, 1.07)	1.04*	(1.01, 1.07)	1.02	(0.99, 1.06)
South	**Male**		**Female**		**Total**	
Factor	**Odds Ratios**	**95% Confidence Intervals**	**Odds Ratios**	**95% Confidence Intervals**	**Odds Ratios**	**95% Confidence Intervals**
Folic Acid Supplementation						
None	*(ref.)*		*(ref.)*		*(ref.)*	
Any	1.98	(0.55, 7.11)	0.73	(0.30, 1.80)	0.95	(0.46, 1.95)
Maternal age (continuous)	1.04	(0.99, 1.09)	1.02	(0.99, 1.06)	1.03	(1.00, 1.06)

*Factor was significant at α=0.05

Table 6: Polytomous logistic regression analysis evaluating factors associated with low spina bifida among all births of women enrolled in the public health campaign by child's sex and folic acid (FA) exposure, People's Republic of China, 1993 to 1996

North	**Male**		**Female**		**Total**	
Factor	**Odds Ratios**	**95% Confidence Intervals**	**Odds Ratios**	**95% Confidence Intervals**	**Odds Ratios**	**95% Confidence Intervals**
Folic Acid Supplementation						
None	*(ref.)*		*(ref.)*		*(ref.)*	
Any	0.64	(0.26, 1.56)	0.46	(0.16, 1.32)	0.54	(0.27, 1.07)
Maternal age (continuous)	1.00	(0.93, 1.08)	1.01	(0.95, 1.09)	1.01	(0.95, 1.06)
South	**Male**		**Female**		**Total**	
Factor	**Odds Ratios**	**95% Confidence Intervals**	**Odds Ratios**	**95% Confidence Intervals**	**Odds Ratios**	**95% Confidence Intervals**
Folic Acid Supplementation						
None	*(ref.)*		*(ref.)*		*(ref.)*	
Any	0.44	(0.13, 1.48)	0.96	(0.48, 1.91)	0.75	(0.41, 1.35)
Maternal age (continuous)	1.00	(0.94, 1.07)	0.89	(0.78, 1.00)	0.95	(0.87, 1.03)