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# A population-based case-control study of risk factors for neural tube defects in four high-prevalence areas of Shanxi province, China

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## Summary

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Shanxi province in Northern China has one of the highest reported prevalence rates of neural tube defects (NTD) in the world. To explore the risk factors for NTDs in Shanxi province, we carried out a population-based case-control study in four selected counties with prevalence rates >10 per 1000 births during 2003. Using a multi-logistic regression model analysis ( $\alpha = 0.10$ ), 158 NTD cases were compared with 226 control mothers.

Maternal factors significantly associated with increased risk for an NTD were a primary school education or lower (adjusted odds ratio [OR] 2.32, 95% confidence interval [CI] 1.09, 4.97); a history of a previous birth defect-affected pregnancy (adjusted OR 5.27, 95% CI 0.98, 28.37); history of a fever or 'cold' (adjusted OR 3.36, 95% CI 1.68, 6.72); use of analgesic and antipyretic drugs (adjusted OR 4.89, 95% CI 0.92, 25.97); daily passive exposure to cigarette smoke (adjusted OR 1.60, 95% CI 0.94, 2.73); poor ventilation during heating (adjusted OR 3.91, 95% CI 0.75, 20.81); and consumption of  $\geq$  six meals per week containing pickled vegetables (adjusted OR 3.86, 95% CI 1.11, 13.47) during pregnancy.

Factors which appeared to be protective were meat consumption one to three times per week (adjusted OR 0.62, 95% CI 0.37, 1.06), or  $\geq$  four times per week (adjusted OR 0.28, 95% CI 0.11, 0.77); and legume consumption  $\geq$  six times per week (adjusted OR 0.39, 95% CI 0.17, 0.89). Differences in risk were found between the two most common phenotypes, anencephaly and spina bifida. Most of the environmental factors had stronger positive and negative associations with risk for anencephaly rather than spina bifida, whereas history of a previous birth defect-associated pregnancy, as well as legume consumption, were more strongly associated with the risk for spina bifida than for anencephaly. The findings suggest that aetiological heterogeneity may exist between anencephaly and spina bifida.

**Keywords:** neural tube defects, prenatal infection, maternal diet, maternal medication, passive smoking, air pollution, environmental exposures.

## Introduction

Neural tube defects (NTD) are among the most common congenital malformations and are a major cause of stillbirth and infant mortality in China where they

account for up to one-third of stillbirths and a quarter to a third of neonatal deaths.<sup>1</sup> Within China, there are differences in NTD prevalence rates between the north, which has a very high rate, and the south, which has

much lower rates, as well as between urban and rural areas, with rural areas reporting higher rates, suggesting that environmental exposure may affect NTD rates in China.<sup>2</sup> It has been suggested that the regional difference in NTD rates may be explained by differential dietary consumption of folate: the southern region of China, which reports lower rates, has a higher socio-economic status, with a more temperate climate and longer growing season than the northern region where folate-rich foods are not available year-round.<sup>3</sup> However, other environmental exposures including environmental pollution and consumption of particular foods may also contribute to the high prevalence rate in some regions.

Shanxi province, a coal mining region in Northern China, has the highest reported birth prevalence of NTDs worldwide, reported to be 10.55 per 1000 births ascertained from hospital-based surveillance in 1987,<sup>2</sup> and 14.9 per 1000 births in 1997 from local government retrospective investigation. These rates were over 10 times higher than those reported from Southern China or the United States during the same period. The reasons for the high NTD rate in Shanxi province is unknown. In order to identify risk factors for the very high incidence in this population, we conducted a population-based case-control study between January 2003 and February 2004 in selected high-prevalence areas of this province.

## Methods

### Study location

The study was conducted in four counties (Pingding, Xiyang, Taigu and Zezhou) in Shanxi province with high rates of NTDs. Our surveillance data indicated that the overall birth prevalence of NTDs in these four counties was 13.9 per 1000 births in 2003, and was >10 per 1000 births in each county.

### Case definition

For this analysis, NTDs included anencephaly, spina bifida and encephalocele. Anencephaly was defined as complete or incomplete occurring either in isolation or in combination with other congenital malformations of various systems; spina bifida was defined as occurring either alone or combined with other congenital malformations of various systems, other than with anencephaly or spina bifida occulta; and

encephalocele was defined as occurring either alone or combined with other congenital malformations of various systems, other than with anencephaly or spina bifida.

### Study subjects

Neural tube defect cases were ascertained from live births, stillbirths and elective terminations that occurred during the 14-month period from 1 January 2003 to 28 February 2004, using a population-based birth defects surveillance system similar to one described previously, which detects external structural defects.<sup>4</sup> When any external malformation was identified, the case was matched to a newborn infant with no identified congenital anomaly by sex, maternal ethnic group, month of conception and county of parents' residence.

### Data collection

Within the first week after delivery, trained health care workers conducted face-to-face interviews with mothers of cases and controls in hospitals, private or community general practice clinics, or at home. Interviewers used a structured questionnaire, and the same interviewer and questionnaire were used for case and control mothers. We collected information on women's sociodemographic characteristics and life style behaviours, as well as history of illnesses, use of medication and oral contraceptives, use of folic acid supplements, potential toxic exposures, environmental exposures, season of conception and the frequency of consumption of certain foods from 1 month before until 2 months after the last menstrual period (LMP) (Table 1). Some paternal exposures were also included in the questionnaire. The study was approved by the Institutional Review Board of Peking University Health Science Centre. All participants provided written informed consent.

### Data management and analysis

Data were entered using the Epi-info 6 statistical package. SPSS (version 10) was used to analyse the data. In order to increase the power of this study, all controls, including controls for NTDs as well as other external malformations, were compared with NTD cases in the analysis. We analysed over 40 suspected hereditary and environmental factors. In univariate analysis, cat-

**Table 1.** Maternal and paternal factors evaluated in determination of risk factors for neural tube defects (NTD) in four counties in Shanxi province

Factor category	Factors
Biological	Consanguinity History of birth defect-affected pregnancy
Social/demographic	Education level (mother) Education level (father) Age (mother) Age (father)
Maternal illness <sup>a</sup>	History of fever or 'cold' History of anaemia History of hepatitis
Medication use <sup>a</sup>	Oral contraceptives Anticonvulsants/sedatives Antibiotics Analgesics/antipyretics (aspirin, aminopyrine, phenacetin)
Toxic or environmental exposures <sup>a</sup>	Pesticides (mother) Pesticides (father) Organic solvents (mother) Organic solvents (father) Heavy metals (mother) Heavy metals (father) Imaging radiation/radiation therapy Kitchen in living quarters Cooking in kitchen Use of coal stove for heating Stove in living room or bedroom Absence of ventilation during heating Presence of heating/cooking fumes in living quarters
Other exposures <sup>a</sup>	Cigarette smoking (mother) Cigarette smoking (father) Passive tobacco smoke Alcohol consumption (mother) Alcohol consumption (father) Tea consumption
Dietary <sup>a,b</sup>	Meat consumption(including seafood) Egg/milk consumption Fresh vegetable consumption Fresh fruit consumption Legume consumption Pickled vegetable consumption Sprouted potato consumption
Folic acid use	<ul style="list-style-type: none"> <li>• Any use during pregnancy</li> <li>• Used between 3 months before and 1 month after last menstrual period (LMP)</li> <li>• Used between 3 months before and 1 month after LMP and continued for at least 1 month</li> </ul>

<sup>a</sup>From 1 month before LMP until 2 months after LMP.

<sup>b</sup>All foods except meat and sprouted potatoes grouped as follows: <1 meal/week, 1–3 meals/week, 4–6 meals/week, and ≥6 meals/week. Meat and sprouted potatoes grouped as follows: <1 meal/week, 1–3 meals/week, and ≥4 meals/week.

egorical variables were analysed using the chi-square test or Fisher exact test. Risks were estimated by the odds ratio (OR), and the precision of the OR was assessed by its 95% confidence interval [CI]. Multivari-

able logistic regression analysis was carried out to evaluate the combined effect of multiple risk factors, adjusting for confounding variables and significant factors. The significance cut-off *P*-value was <0.05 in

**Table 2.** Demographic characteristics of case and control parents

Characteristics	Cases (N = 158)		Controls (N = 226)		OR [95% CI]
	n	(%)	n	(%)	
Age at interview (years)					
<25	64	(40.5)	90	(39.8)	1.00 Reference
25–29	50	(31.6)	86	(38.1)	0.82 [0.51, 1.31]
30–34	38	(24.1)	39	(17.3)	1.37 [0.79, 2.37]
35+	5	(3.2)	8	(3.5)	0.88 [0.28, 2.81]
Missing/unknown	1	(0.6)	3	(1.3)	–
Highest level of education					
High school or higher	12	(7.6)	28	(12.4)	1.00 Reference
Junior high school	118	(74.7)	178	(78.8)	1.55 [0.76, 3.16]
Primary school or lower	28	(17.7)	17	(7.5)	3.84 [1.55, 9.50]
Missing/unknown	0	(0.0)	3	(1.3)	–
Father's age (years)					
<25	38	(24.1)	52	(23.0)	1.00 Reference
25–29	64	(40.5)	109	(48.2)	0.80 [0.48, 1.35]
30–34	41	(25.9)	47	(20.8)	1.19 [0.66, 2.16]
35+	12	(7.6)	14	(6.2)	1.17 [0.49, 2.82]
Missing/unknown	3	(1.9)	4	(1.8)	–
Father's highest level of education					
High school or higher	11	(7.0)	28	(12.4)	1.00 Reference
Junior high school	131	(82.9)	186	(82.3)	1.79 [0.86, 3.73]
Primary school or lower	16	(10.1)	11	(4.9)	3.70 [1.31, 10.44]
Missing/unknown	0	(0.0)	1	(0.4)	–

OR, odds ratio; CI, confidence interval.

the univariate analysis. Considering the relatively small sample size for multivariable analysis, we use  $P < 0.10$  as the cut-off.

## Results

### Participation rate

A total of 181 NTD cases were identified during the study period. Among these, the mothers of 158 (87.3%) agreed to participate, and completed the interview. The 158 NTD cases included 49 (31.0%) elective terminations and 109 live births or stillbirths. Among all NTD cases, there were 71 (44.9%) cases of anencephaly, 75 (47.5%) cases of spina bifida and 12 (7.5%) cases of encephalocele.

Among 238 contacted controls, 227 (95.4%) mothers completed the interview. We excluded one control because the mother was of the Hui minority ethnicity and all of the other case mothers were of Han ethnicity (the predominant ethnic group in China). This left 226 eligible (95.0%) controls.

**Table 3.** Significant maternal illnesses, drug use, and exposures among case mothers between 1 month before and 2 months following conception, compared with control mothers

Factor	Cases <sup>a</sup> (N = 158)	Controls <sup>a</sup> (N = 226)	OR [95% CI]
History of fever or cold			
No	112	203	1.00 Reference
Yes	40	20	3.63 [2.02, 6.50]
Anticonvulsant or sedatives use <sup>b</sup>			
No	152	225	
Yes	5	0	–
Antibiotic use			
No	133	211	1.00 Reference
Yes	25	12	3.31 [1.61, 6.80]
Analgesic and antipyretic drug use			
No	145	221	1.00 Reference
Yes	12	2	9.15 [2.02, 41.46]

OR, odds ratio; CI, confidence interval.

<sup>a</sup>Values for some characteristics may not be equal to total numbers of case or control groups because of missing values.

<sup>b</sup>Difference was significant with Fisher exact test.

**Table 4.** Dietary factors during periconception in case and control women

Factors	Cases <sup>a</sup> (N = 158)	Controls <sup>a</sup> (N = 226)	OR [95% CI]	P <sup>b</sup>	P <sup>c</sup>
<b>Meat consumption</b>					
<1 meal per week	102	108	1.00 Reference		
1–3 meals per week	45	84	0.34 [0.13, 0.87]	<0.001	<0.001
≥4 meals per week	6	33	0.19 [0.08, 0.48]		
<b>Egg or milk consumption</b>					
<1 meal per week	51	71	1.00 Reference		
1–3 meals per week	60	81	1.03 [0.63, 1.69]	0.73	0.51
4–6 meals per week	27	50	0.75 [0.42, 1.36]		
>6 meals per week	16	24	0.93 [0.45, 1.92]		
<b>Fresh vegetable consumption</b>					
<1 meal per week	12	30	1.00 Reference		
1–3 meals per week	34	55	1.54 [0.70, 3.42]	0.27	0.12
4–6 meals per week	53	65	2.04 [0.95, 4.36]		
>6 meals per week	56	75	1.87 [0.88, 3.96]		
<b>Fresh fruit consumption</b>					
<1 meal per week	29	37	1.00 Reference		
1–3 meals per week	51	59	1.10 [0.60, 2.04]	0.19	0.41
4–6 meals per week	30	64	0.60 [0.31, 1.15]		
>6 meals per week	47	65	0.92 [0.50, 1.71]		
<b>Legume consumption</b>					
<1 meal per week	63	67	1.00 Reference		
1–3 meals per week	56	73	0.82 [0.50, 1.33]	<0.01	<0.001
4–6 meals per week	26	49	0.56 [0.31, 1.02]		
>6 meals per week	10	35	0.30 [0.14, 0.67]		
<b>Pickled vegetable consumption</b>					
<1 meal per week	83	143	1.00 Reference		
1–3 meals per week	49	63	1.34 [0.85, 2.13]	<0.01	<0.002
4–6 meals per week	13	12	1.87 [0.81, 4.28]		
>6 meals per week	11	3	3.16 [1.13, 8.85]		
<b>Sprouted potato consumption</b>					
<1 meal per week	117	175	1.00 Reference		
1–3 meals per week	25	35	1.07 [0.61, 1.88]	0.44	0.29
≥4 meals per week	9	7	1.92 [0.70, 5.31]		

OR, odds ratio; CI, confidence interval.

<sup>a</sup>Values for some characteristics may not be equal to total numbers of case or control groups because of missing values.

<sup>b</sup>Global test for a difference in risk among categories.

<sup>c</sup>Test for a linear trend in risk.

### Univariate analysis for risk factors

The mean ages of case and control mothers were 27.1 and 26.6 respectively, and the age distributions of mothers were similar in both groups, with approximately three-quarters being under age 30 (Table 2). The mean ages of case and control fathers were 28.8 and 28.3 respectively, and their age distributions were also similar. Case mothers and fathers were more likely than control parents to have a primary school or lower level of education (Table 2).

Consanguinity between parents or grandparents was infrequent in both cases and controls (one control couple; and one case and one control set of grandparents) and was not associated with an increased likelihood of NTDs. Mothers of cases were significantly more likely to have had a previous birth defects-affected pregnancy (9/146) than were mothers of controls (2/224) (OR 6.90, 95% CI 1.47, 32.41). Cases were more likely than controls to report a history of fever or cold, anticonvulsant or sedative use, antibiotic use, and analgesic or antipyretic use during this period

**Table 5.** Reported use of folic acid supplements before and during early pregnancy by case and control mothers, Shanxi province

	Case mothers <sup>a</sup> (N = 158)		Control mothers <sup>a</sup> (N = 226)		OR [95% CI]
	n	(%)	n	(%)	
Any folic acid use					
No	141	(89.2)	207	(91.6)	1.00 Reference
Yes	17	(10.8)	19	(8.4)	1.31 [0.66, 2.62]
Used between 3 months before and 1 month after LMP					
No	152	(96.2)	218	(96.5)	1.00 Reference
Yes	6	(3.8)	8	(3.5)	1.08 [0.37, 3.16]
Used between 3 months before and 1 month after LMP and continued for at least 1 month					
No	153	(97.5)	220	(97.3)	1.00 Reference
Yes	4	(2.5)	6	(2.7)	0.96 [0.27, 3.45]

OR, odds ratio; CI, confidence interval; LMP, last menstrual period.

<sup>a</sup>Values for some characteristics may not be equal to total numbers of case or control groups because of missing values.

(Table 3); however, other illnesses and potential toxic exposures were similar among case and control mothers. Paternal factors were not significantly associated with an increase in NTD risk.

While only four case mothers (2.6%) and four control mothers (1.8%) reported smoking during the periconceptional period, 127 of 158 (80.4%) case fathers and 163 of 226 (72.1%) control fathers smoked cigarettes. Neither maternal nor paternal smoking was significantly more prevalent among cases or controls; however, daily exposure to passive cigarette smoke was reported for 52 of 154 (33.8%) case mothers and 52 of 223 (23.3%) control mothers, and was significantly

associated with having an NTD-affected pregnancy (OR 1.68, 95% CI 1.06, 2.65). More than 80% of case and control mothers reported that they had a separate kitchen and a similar proportion reported that most cooking was done in the kitchen; neither of these factors was associated with NTD risk, nor was the use of a stove for heating (37.6% of case mothers, 33.5% of control mothers) or the presence of a stove in the living room or bedroom (29.9% of case mothers, 23.3% of control mothers). However, the absence of adequate ventilation during heating was significantly associated with having an NTD-affected pregnancy (11 of 158 [7.0%] case households vs. 2 of 226 [0.9%] control

**Table 6.** Multivariable logistic regression – significant risk factors for all NTDs, anencephaly and spina bifida ( $\alpha = 0.10$ )

Risk factor	Overall		Anencephaly (N = 71)		Spina bifida (N = 75)	
	OR	[95% CI]	OR	[95% CI]	OR	[95% CI]
Primary school education or less (mother)	2.32	[1.09, 4.97]	3.03	[1.18, 7.81]	2.71	[1.14, 6.44]
Previous history of birth defect-affected pregnancy	5.27	[0.98, 28.37]	–	–	7.34	[1.38, 39.19]
Fever or cold	3.36	[1.68, 6.72]	3.72	[1.63, 8.51]	3.51	[1.61, 7.68]
Analgesic and antipyretic drug use	4.89	[0.92, 25.97]	9.97	[1.76, 56.46]	–	–
Daily exposure to passive cigarette smoke	1.60	[0.94, 2.73]	1.88	[0.92, 3.73]	–	–
Inadequate ventilation during heating	3.91	[0.75, 20.81]	–	–	–	–
Pickled vegetable consumption ( $\geq 6$ meals per week)	3.86	[1.11, 13.47]	5.46	[1.61, 18.45]	–	–
Meat consumption ( $\geq 4$ meals per week)	0.28	[0.11, 0.77]	0.23	[0.05, 1.05]	–	–
Meat consumption (1–3 meals per week)	0.62	[0.37, 1.06]	0.39	[0.19, 0.84]	–	–
Legume consumption ( $\geq 6$ meals per week)	0.39	[0.17, 0.89]	–	–	0.09	[0.01, 0.70]

NTD, neural tube defect; OR, odds ratio; CI, confidence interval.



households; OR 8.38, 95% CI 1.83, 38.36). Half of case and control mothers became pregnant with the index pregnancy during the winter months.

Investigation of dietary factors showed that increasing frequency of consumption of meat and legumes was associated with a protective effect, which increased with increasing dose (Table 4). Consumption of pickled vegetables, on the other hand, carried dramatically increased risk with clear linear trends. No obvious association was found for consumption of eggs or milk, fresh vegetables, fresh fruits and sprouted potatoes.

Reported use of any folic acid supplements before and during early pregnancy was low, and was similar among cases (10.8%) and controls (8.4%) (Table 5). High compliance (using between 3 months before and 1 month after LMP, or using between 3 months before and 1 month after LMP and continuing for at least 1 month) was lower: 3.8% and 2.5% for case mothers, and 3.5% and 2.7% for control mothers respectively.

### **Multivariable analysis**

A multivariable logistic model of risk was constructed using a backward stepwise procedure by adding all the significant risk factors from the univariate analysis. The multilevel factors such as education level and dietary factors were put into models by transforming them into several dichotomous variables. The final model confirmed the significance (exclusion cut-off *P*-value: 0.10) of 10 risk factors (shown in Table 6). The effects of father's education, the use of anticonvulsants or sedatives, and the use of antibiotics disappeared in the final model, with adjustment for other important variables.

### **Risk comparison between anencephaly and spina bifida**

We further constructed two logistic models using a backward stepwise procedure by adding significant factors from the multivariable analysis to determine whether there were differences in risk for the two most common NTD phenotypes, i.e. anencephaly and spina bifida (Table 6). We found that compared with spina bifida, anencephaly risk was more strongly associated with the use of analgesics and antipyretics, daily passive smoke exposure, and consumption of six or more pickled vegetable servings per week, and that consumption of one to three meat meals per week was

protective. History of a previous birth defect-affected pregnancy was associated with risk for spina bifida, but not for anencephaly.

### **Discussion**

In this population-based case-control study of risk factors for NTDs in a high incidence area of China, we found that increased risk of an NTD-affected pregnancy was associated with a low level of maternal education; previous history of a pregnancy complicated by a birth defect; history of a fever or 'cold' during early pregnancy, use of anticonvulsants, sedatives, antibiotics, analgesics and antipyretics before and during early pregnancy; daily exposure to passive cigarette smoke; and consumption of pickled vegetables. Consumption of meat and legumes appeared to be protective. Furthermore, the increased risk associated with a previous birth defect-affected pregnancy was seen only for spina bifida, and not for anencephaly.

An increased risk of recurrence following an index NTD-affected pregnancy has been well-described.<sup>5-8</sup> Although we did not collect information on the type of birth defect that occurred in previous pregnancies, we found approximately a fivefold increased risk of having a spina bifida-affected pregnancy among women who had a history of a previous birth defect.

Risk for NTDs was inversely related to the level of maternal education, with women who had a primary school education or less having more than a twofold risk, compared with those with higher education. A similar association with low education was found in a US study,<sup>9</sup> and a number of studies have reported an association between low socio-economic status and the risk for NTDs,<sup>10</sup> in part due to lack of awareness of the potential benefit of folic acid supplements in preventing NTDs, or of the importance of periconceptional consumption of folic acid among women with less education.<sup>11</sup> While our study supports previous studies showing a correlation between low socio-economic status and risk of NTD-affected pregnancy, the overall use of folic acid in this study population was extremely low, suggesting that other factors in addition to knowledge of folic acid, such as dietary habits, access to medical care or other unknown factors, may be associated with lower education.

Numerous human epidemiological studies implicated maternal hyperthermia, or fever-producing illnesses, including the common cold, as risk factors for NTDs.<sup>12-17</sup> Data from animal studies also suggest that



hyperthermia itself is the teratogen.<sup>18</sup> We found that a history of fever or cold during the periconceptional period was associated with approximately a threefold increased NTD risk, which persisted after controlling for other relevant covariates. Because a cold is a common source of fever, and because fever and colds often occur concomitantly, we investigated them as one item in the questionnaire, and therefore could not determine from the current data whether the observed increased risk was attributable to the elevated temperature or to the cold.

We found that the use of analgesic and antipyretic drugs was associated with an increased risk of having a NTD-affected pregnancy. These drugs were commonly used to relieve pain and reduce fever, and their use was highly correlated with a history of fever or cold (data not shown,  $P < 0.001$ ). However, even after controlling for cold or fever in a multivariable model, the use of these drugs was still significantly associated with an increased risk. There are few previous data to support this finding: one animal study showed that while aspirin alone did not result in embryotoxicity, its administration potentiated hyperthermia-induced teratogenesis in the mouse.<sup>19</sup> A meta-analysis found no evidence of an overall increase in the risk of congenital malformations that could be associated with aspirin.<sup>20</sup> Aspirin exposure during the first trimester may be associated with an increased risk of gastroschisis.<sup>20</sup> Therefore, the significance of our finding is unclear, and deserves further investigation.

There are few data regarding the association between NTD risk and parental cigarette smoking, or exposure to passive cigarette smoke. We found a moderately increased risk of NTDs associated with daily maternal exposure to passive cigarette smoke, which was supported by an animal study from China which indicated that passive smoking could induce murine spina bifida, and exencephaly during neurulation.<sup>21</sup> However, Wasserman *et al.*<sup>22</sup> reported that parental smoking was not associated with an increased risk of NTDs based on a case-control study, and Kallen<sup>23</sup> reported a significant, protective effect of maternal smoking on the incidence of NTDs.

Nutritional factors have been implicated in the aetiology of NTDs;<sup>24</sup> deficiency of protein, vitamins and other micronutrients,<sup>25–27</sup> most notably, folate<sup>28,29</sup> have been associated with an increased risk of NTDs. In this study, frequent consumption of meat (though not other animal proteins such as milk and eggs) and legumes was associated with a decreased risk of NTDs, while

consumption of fresh fruit and vegetables did not appear to affect the risk. Meat and fish provide not only protein, but also other nutrients, including folic acid and minerals. Despite the fact that many of the families in this study kept some livestock for selling, the consumption of meat was relatively uncommon; in rural areas of Northern China, the staple foods are cereals, grains and root crops, with legumes being a substantial source of dietary protein. In addition, germinated or sprouted legumes, which are rich in vitamin C and, in some instances, riboflavin and niacin,<sup>30</sup> are often added to foods to increase their nutrient content.

We also found by Spearman's correlation analysis a correlation between the frequency of consumption of meat, with that of eggs, dairy products, fresh fruit and legumes, and between consumption frequency of legumes with that of eggs, dairy products, fresh fruit and meats, but no correlation between consumption frequency of fresh vegetables, pickled vegetables and sprouted potato with that of meats and legumes (data not shown). Therefore, the apparent protective effects of meat and legume consumption may include some effect from consumption of eggs, dairy products, fresh fruit, or a combination of these.

Our study revealed a significant association between periconceptional consumption of pickled vegetables and an increased risk of having an NTD-affected pregnancy, and a dose-response relationship between weekly consumption frequency and NTD risk. Pickled vegetables contain high concentrations of N-nitroso compounds;<sup>31</sup> several studies from England, Australia, Canada, China and the United States have suggested that maternal exposure to nitrates from drinking water or other dietary sources may increase the risk of congenital malformation.<sup>32–36</sup> Previous studies from this province have shown a spatially consistent distribution of prevalence rates of central nervous system malformations with the death rate from malignant carcinoma, indicating that the same environmental risk factors may be shared by congenital malformations and malignant carcinoma.<sup>37</sup> Shanxi province is among the high-risk areas for oesophageal cancer in China, and consumption of nitrates and nitrites from pickled vegetables have been proposed as important risk factors for oesophageal cancer in China.<sup>38,39</sup> Hence, N-nitroso compounds from pickled vegetables may play a role in the aetiology of both of these conditions.

Shanxi province possesses abundant coal reserves. Coal mining is a major industry, and the burning of

coal in stoves for cooking and heating is widespread, resulting in substantial indoor air pollution, particularly in rural kitchens and bedrooms. To examine the role of indoor air pollution in the aetiology of NTDs, several related items were analysed in the questionnaire; however, only inadequate ventilation during heating was found to be associated with an increased risk for NTDs. Because exposure to indoor air pollution from coal combustion is so widespread in the area, we were not able to identify a differential exposure between case and control households. However, further studies about its relationship to NTD risk are warranted.

It is well known that daily periconceptional supplementation with folic acid supplementation is effective in reducing the recurrence and occurrence of NTDs.<sup>40–42</sup> We tried to determine whether compliance with the recommendations for folic acid use before and during early pregnancy affected the risk for NTDs by looking at different times of starting and stopping folic acid relative to the LMP; however, the overall low use (10%) of folic acid supplements in the project area precluded our identifying an association between folic acid use and the risk of NTDs.

Our comparison analysis indicated that risk factors for anencephaly were different from those for spina bifida. Anencephaly was significantly associated with most of the environmental factors including analgesic and antipyretic drug use, daily exposure to passive cigarette smoke, and pickled vegetables consumption, with meat consumption being protective, whereas spina bifida was associated with a history of a previous birth defect-affected pregnancy. These findings may support the hypothesis of aetiological heterogeneity among NTDs.<sup>43,44</sup>

Our study has several strengths. The study was conducted in a very high NTD-prevalence area, increasing the likelihood of identifying important risk factors. Selection bias was minimised by the population-based design and high response rate of this study. Ascertainment was facilitated by the fact that NTDs are major external congenital anomalies that are easily identified by the existing birth defects surveillance system, and not easily missed or confused with other defects; cases were definitively diagnosed by three paediatricians at Peking University, using clinical descriptions and standard photographs. Therefore, we feel the risk of misclassification to be almost non-existent.

One important potential source of bias could be recall bias, with case mothers tending to provide more

information about items they believe to be risk factors. However, to the best of our knowledge, the local residents have little knowledge about the existence of environmental risk factors for NTDs, particularly related to pickled vegetables consumption and indoor air pollution. Because consumption of pickled vegetables is common throughout the study area, and was not perceived to be potentially causally related to the occurrence of birth defects, we feel that recall bias was unlikely to have resulted in the differential reporting between case and control mothers. In addition, the differences in risks for the two NTD phenotypes that we observed may provide indirect evidence supporting a causal relationship, as there is little reason to assume that anencephaly case mothers would report pickled vegetables consumption less accurately than spina bifida case mothers. Hence, recall bias is unlikely to explain our results.

The most important potential limitation of our study may be the small sample size; some variables were analysed for very few women, with some zero cells. To increase the power, all controls (including controls of NTD cases and other congenital anomalies) were compared with the NTD cases in analysis. In addition, because we were testing so many factors, it is possible that some of our findings may be attributable to chance. However, as so little is known about NTD risk factors in this very high prevalence population, the primary aim of our study was to screen risk factors and generate hypotheses for further in-depth study. Despite these limitations, the risk factors identified in this study may help other researchers to conduct additional aetiological studies and to devise more comprehensive strategies for the prevention of NTD in China and throughout the world.

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