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Maternal periconceptional factors affect the risk of spina bifida-affected pregnancies: an Italian case–control study

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Abstract
Purpose Neural tube defects, including spina bifida and anencephaly, are the second most common birth defects with an incidence in Italy of 0.4–1/1,000. Information on factors playing a role in the pathogenesis of spina bifida is based on populations with different exposures, lifestyle, social and cultural habits compared to Italian people. Our objective was to fill this gap by using data from a case–control interview study carried out at the G. Gaslini Children's Hospital, Genoa, from 2000 to 2008.
Methods We surveyed questionnaires from 133 case mothers and 273 control women providing information on periconceptional risk factors. Univariate and multivariate logistic regression analyses were used to estimate risks by odds ratios (ORs) and 95% confidence intervals (95% CIs).
Results Univariate results suggest that birth order, low maternal educational level, age, smoking habits, alcohol consumption, high caffeine intake, lack of folate supplementation, low and high calorie diet, occasional consumption of fruit and vegetables, high emotional stress, and environmental pollution are associated with an increased spina bifida risk. Nevertheless, high caffeine intake (OR=10.82; 95% CI, 3.78–31), low calorie diet (OR=5.15; 95% CI, 1.79–14), occasional consumption of fruit and vegetables (OR=3.38; 95% CI, 1.67–6.82), alcohol consumption (OR=3.05; 95% CI, 1.24–7.50) and, above all, lack of folate supplementation at any time of pregnancy (OR=20.54; 95% CI, 5.41–77) mainly determined spina bifida risk in the multivariate analysis.
Conclusion Our findings point out that a common underlying mechanism, a disturbed folate/homocysteine metabolism, may be causative for the burden of spina bifida in the Italian population.

Keywords Neural tube defects (NTD) · Spina bifida · Case–control study · Lifestyle · Periconceptional risk factors

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Introduction

Neural tube defects (NTD) are among the most common human congenital malformations caused by partial or complete failure of fusion in the cranial (e.g., anencephaly and encephalocele) and spinal regions of the neural tube during early embryogenesis [2]. Defects involving spinal cord and vertebral arches represent a clinical spectrum and are referred to as spinal dysraphisms [38]. Spinal dysraphisms may be categorized clinically into open and closed, based on whether the abnormal nervous tissue is exposed to the environment or covered by skin. Myelomeningocele, also called spina bifida, is the most common type of spinal dysraphism, accounting for about 90% of all spinal defects [38].

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Rates of NTD vary greatly across time and among different geographic areas at the same time. The highest incidences have been reported among Mexican, Irish, Indian, and Northern China populations [2, 21, 31, 48]. A Western Europe surveillance network (EUROCAT) has provided prevalence data for birth defects in eighteen European countries since 1980 [11]. The incidence of NTD in European countries excluding UK and Ireland was only 0.1–0.6 per 1,000 births in the period 1989–2002 [3]. In Italy (an area with relatively low rates), when live births, stillbirths, and induced termination of pregnancy are considered, the prevalence was 0.4–1 in 1,000 newborns [3]. Approximately 530,000 pregnancies per year occurred in Italy, resulting in 370 new cases.

Most NTD appear to be multifactorial, with environmental factors interacting with polygenic predisposition. In particular, maternal nutritional factors have been identified as important contributors [17]. The best known of these factors is the preventive role of supplemental folic acid [8, 28]. In Italy, the relatively low prevalence of NTD, together with some underestimation of the social weight of birth defects by the national health service, may have contributed to the delay in establishing approaches for the prevention of NTD. In 2004, the Italian Network for Folic Acid Promotion was organized and, coordinated by the National Center for Rare Diseases of the Italian National Institute of Health, developed and diffused a recommendation promoting an increased intake of folic acid (0.4 mg/day) by women in fertile age (http://www.cnmr.iss.it). The Italian Ministry of Health in the Drugs Bulletin published and distributed the draft recommendation to family practitioners and specialists.

Besides the role of folic acid, a number of maternal factors have also been found implicated in the etiology of NTD, particularly hyperthermia, diabetes, hyperinsulinemia, obesity, psychosocial or emotional stress [25, 30, 32, 43, 44, 49]. Maternal epilepsy and medications used to treat epilepsy, valproic acid and carbamazepine, have been also found to increase the risk of spina bifida [10, 13, 19, 22]. Other factors are still mostly speculative and need to be evaluated further [7, 9, 24, 37, 41, 51].

Most of the currently available information on environmental risk factors playing a role in the pathogenesis of spina bifida is based on populations with different exposures, lifestyle, social and cultural habits compared to those of Italian people. More specifically, the incidence in one geographic area may differ because of different exposures to a given risk factor, or because of differences among populations in their genetic susceptibilities to that risk factor. So far, in Italy, no studies have been carried out with the specific aim of investigating the possible correlation between spina bifida and maternal exposures and lifestyle. Yet, many studies have focused on congenital anomalies in general.

To fill this gap, we performed a hospital-based case–control study to identify maternal periconceptional health and lifestyle factors that may affect the risk of having a child with spina bifida in Italy.

Methods

Study population

A case–control study was conducted between March 2000 and January 2008 at the G. Gaslini Institute (IGG) in Genoa, the largest children's hospital in Italy, having a wide referral base, especially from the South of Italy. Since its founding in 1976, the Neurosurgery Department of IGG has grown substantially in patient volume and become a major national referral center for NTD patients. The study design, the consent form, and the interview instrument were approved by IGG Ethics Committee.

From 2000 to 2008, 850 NTD families were in active follow-up at the Neurosurgery Department of IGG. As eligible cases, we selected mothers who met the following inclusion criteria: (1) they were white Caucasian Italians, (2) they gave information within 24 months after the delivery of the index pregnancy: this time frame was chosen since most malformations are identified during the first to second years of life, which is important for the proper selection of controls, and this cut-off value minimizes the risk of recall bias, and (3) they had a child with non-syndromic open or closed spinal dysraphism. Information on the child's diagnosis was obtained from the clinical record. One hundred forty-five mothers met the above inclusion criteria, and 133 (92%) of them agreed to participate and completed the entire questionnaire. The proband's diagnosis was the following: open spinal dysraphisms (myelomeningocele, 49.5%) and closed spinal dysraphisms (51.5%). Control mothers were enrolled prospectively in the same period as the cases, among Caucasian mothers who were admitted to the IGG for miscellaneous illnesses of their child (orthopedic, otolaryngeal, odontologic, obstetric, acute surgical conditions, and trauma). Inclusion criterion was no family history of birth defects. For control mothers whose children were born at IGG (64%), eligibility was confirmed by checking the birth registration forms. Controls were matched for age at delivery (±1–2 years), sex of index child, and geographical residence area. Of the 332 control mothers who were asked to participate, 273 control mothers agreed to participate.

Questionnaire

With a five-page instrument covering 26 items, the interviewer solicited information on all relevant exposure

and lifestyle during the 3 months before until 3 months after conception (periconceptional period). In fact, we wanted to explore the role of risk factors presented in the most recent literature. A pre-test of the questionnaire identified patient concerns regarding the purpose of the study. Revisions to the questionnaire were made based on these problems and concerns. The same structured questionnaire was administered to both cases and controls by two trained interviewers. Interviews were collected during average of 20 months for NTD mothers and 18 months for control mothers after the date of delivery. The questionnaire covered the period of 3 months before until 3 months after conception. It consisted of four parts: (1) a demographic section that gathered information on mother's birth date, date of delivery, country of birth, educational level (years of schooling), annual family income, marital status and consanguinity with the father; (2) a section on reproductive history (miscarriages and termination of pregnancy for fetal abnormality), pregnancy history (birth order, age at delivery, folic acid supplementation, and pregnancy complication); and (3) a section on lifestyle and exposures.

Lifestyle habits included smoking, alcohol, fruit and vegetable consumption, caffeine intake, unbalanced diet, and emotional stress. Exposures included medication use, multivitaminic and iron therapies, radiation, toxics, and environmental pollution due to the residence near waste disposal sites or contaminated lands. Maternal smoking was categorized as no smoker (never smoker or ex-smoker since the last year before conception) and current smoker. To assess caffeine and alcohol consumption, women were asked to report the number of coffee cups (more or less than three cups/day) and the amount of alcohol (less or more than half a liter) drunk per day. To assess fruit and vegetable consumption, women were asked to estimate the number of times per week they ate fruit and vegetables (regular, three or more times/week; occasional, less than three times/week). Low calorie diet was defined as a diet of less than 1,200 calories per day, normal diet as providing 1,200–2,000 calories, and high calorie diet, more than 2,000 calories.

Statistical analysis

Descriptive statistics were generated for the whole cohort and data were expressed as mean and standard deviation for continuous variables. Moreover, median value and range were calculated and reported, as were absolute or relative frequencies for categorical variables.

Univariate analysis was carried out to determine which of the various potential risk factors in the case population were significantly associated with the risk of spina bifida. Logistic regression analyses were used for each variable, and the results are reported as odds ratio (OR) with their 95% confidence interval (CI).

In the case of zero cells in the tables, 0.5 was added to estimate real values.

Multivariate analysis was then performed, and only variables that proved to be statistically or borderline significant in univariate analysis were included in the model. In this case, a P value<0.05 was used as the cut-off. The variables included in the initial model were educational level, birth order, maternal age, smoking habits, medium and high caffeine intake, alcohol intake, fruit and vegetable consumption, diet, folic acid supplementation, emotional stress, and residency near waste sites. Statistical analysis was performed using SPSS for Windows (SPSS Inc, Chicago, IL).

Results

Mean maternal age at delivery was comparable in the two groups (case mothers, 29.1 years; control mothers, 29.3 years). Table 1 shows the results of univariate analysis of maternal characteristics and spina bifida risk. Maternal low educational level (0–8 years of schooling) proved to be a risk factor for spina bifida (OR=4.87; 95% CI, 2.38–9.97) as well as the median annual family income (OR=3.35; 95% CI, 1.34–8.34). Low (<20,000 euros) family income, consanguinity with the partner, and previous history of spontaneous abortions did not prove to be spina bifida risks. Being single or divorced (OR=0.23; 95% CI, 0.07–0.78) or otherwise not married (OR=0.27; 95% CI, 0.10–0.70) proved to have an apparent protective effect. Risk factors for spina bifida related to pregnancy history are also listed in Table 1. Birth order was a significant risk with two- and threefold higher risk if the index case was second- (OR=2.15; 95% CI, 1.25–3.69) or third- (OR=3.93; 95% CI, 1.69–9.17) born, respectively. A U-shaped relationship between maternal age and spina bifida risk was found, with an increased risk in the lowest age group (less than 25 years; OR=3.36; 95% CI, 1.89–5.36) and in the highest age group (over 35 years; OR=5.21; 95% CI, 2.42–11). The risk was not substantially influenced by weight gain during pregnancy, by acute diseases that occurred during pregnancy, such as infections, diabetes, hypertension, anemia, or by precocious complications such miscarriage threat. Regarding folic acid supplement use, women who never used folic acid had a significant upsurge in the risk (OR=27; 95% CI, 9.31–78). A significant risk was also found for women using folic acid (0.4 mg/day) after conception (OR=3.35; 95% CI, 1.14–9.84). Table 2 reports the association between maternal periconceptional and first-trimester exposures and spina bifida risk. For some agents, the results of univariate analyses showed a strong but not highly significant association with spina bifida risk, such as the use of antipyretics (OR=10.61; 95% CI, 1.23–91) and

Table 1 Univariate analysis of the association between maternal factors and the NTD risk

Variable	Cases (*N*=133; %)	Controls (*N*=273; %)	OR (95% CI)	*P* value
Education level (years)[a]				
0–8	42 (45.1)	51 (18.8)	4.87 (2.38–9.97)	<.001
9–13	38 (40.9)	143(52.8)	1.57 (0.79–3.13)	
>13	13 (14)	77 (28.4)	Ref.	
Family annual income (euro)[b]				
<20,000	23 (24.7)	80 (33.5)	1.82 (0.68–4.84)	.008
20–40,000	64 (68.8)	121 (50.6)	3.35 (1.34–8.34)	
>40,000	6 (6.5)	38 (15.9)	Ref.	
Marital status[c]				
Divorced/separated/never married	4 (3.2)	30 (11)	0.23 (0.07–0.78)	.002
Co-habiting	5 (5.3)	43 (15.8)	0.27 (0.10–0.70)	
Married	86 (91.5)	199(73.2)	Ref.	
Consanguinity[d]				
No	129 (97)	221 (97.4)	Ref.	
Yes	4 (3)	6 (2.6)	1.14 (0.32–4.12)	.84
Spontaneous abortions[e]				
No	110 (82.7)	196 (72.6)	Ref	
Yes	23 (17.3)	74(27.4)	0.55 (0.33–0.93)	.03
Birth order[f]				
1	49 (52.1)	178 (73)	Ref.	
2	32 (34)	54 (22.1)	2.15 (1.25–3.69)	
≥3	13 (13.9)	12 (4.9)	3.93 (1.69–9.17)	.001
Age at delivery[g]				
<25 years	35 (26.3)	26 (11.6)	3.36 (1.89–5.36)	
25–35 years	75 (56.4)	187 (83.5)	Ref.	<.001
>35 years	23 (17.3)	11 (4.9)	5.21 (2.42–11)	
Weight gain during the pregnancy[h]				
<8 kg	13 (14.4)	27 (13)	1.04 (0.50–2,15)	
8–16 kg	63 (70)	136 (65.4)	Ref.	.482
>16 kg	14 (15.6)	45 (21.6)	0.67 (0.34–1.31)	
Folic acid supplements[i]				
Before and after conception	4 (3)	61 (22.3)	Ref.	
After conception	35 (26.3)	159 (58.3)	3.35 (1.14–9.84)	
Never	94 (70.7)	53 (19.4)	27 (9.31–78)	<.001
Infections[j]				
No	113 (85)	202 (74.8)	Ref.	
Yes	20 (15)	68 (25.2)	0.53 (0.30–0.91)	.022
Anemia				
No	122 (91.7)	248 (90.8)	Ref.	
Yes	11(8.3)	25 (9.2)	0.89 (0.42–1.87)	.76
Hypertension				
No	123 (92.5)	263 (96.3)	Ref.	
Yes	10 (7.5)	10 (3.7)	2.14 (0.87–5.27)	.099
Diabetes				
No	131 (98.5)	270 (98.9)	Ref.	
Yes	2 (1.5)	3 (1.1)	1.37 (0.23–8.32)	.73
Miscarriage threat				
No	118 (88.7)	241 (88.3)	Ref.	
Yes	15 (11.3)	32 (11.7)	0.96 (0.50–1.84)	.896

Table 1 (continued)

Variable	Cases (*N*=133; %)	Controls (*N*=273; %)	OR (95% CI)	*P* value
Other pregnancy complications				
No	98 (73.7)	189 (69.2)	Ref.	
Yes	35 (26.3)	84 (30.8)	0.80 (0.50–1.28)	.355

OR odds ratio, *CI* confidence interval, *Ref.* reference group

[a]Data not available for 40 NTD mothers and 2 control mothers

[b]Data not available for 40 NTD mothers and 34 control mothers

[c]Data not available for 38 NTD mothers and 1 control mother

[d]Data not available for 46 control mothers

[e]Data not available for 3 control mothers

[f]Data not available for 39 NTD mothers and 29 control mothers

[g]Data not available for 49 control mothers

[h]Data not available 43 NTD mothers and 65 control mothers

[i]Both NTD and control mothers used a minimum of 400 micrograms of folic acid per day

[j]Data not available for 3 control mothers

pesticides or solvents (OR=10.62; 95% CI, 1.23–91). No association was found for other medical exposures, and no significant protective effect was demonstrated for the use of multivitaminics and iron therapies. A significantly higher risk was observed in association with the residence near waste sites or polluting industries (OR=3.57; 95% CI, 1.54–8.29). Table 3 summarizes the results of univariate analysis on the association between maternal lifestyle factors and spina bifida risk. The strongest association was observed for high (more than three cups per day) caffeine intake (OR=7.78; 95% CI, 4.02–15.05), low calorie diet (OR=4.63; 95% CI, 2.29–9.33), and occasional (less than three times per week) consumption of fruit and vegetables (OR=4.68; 95% CI, 2.83–7.74). Not so high but statistically significant values were found for smoking habits (OR=1.91; 95% CI, 1.16–3.14), high emotional stress (OR=2.58; 95% CI, 1.47–4.52), high calorie diet (OR=3.31; 95% CI, 1.70–6.45), and alcohol intake (OR=3.69; 95% CI, 2.12–6.42). The multivariate model (Table 4) revealed that the main determinants for spina bifida risk were high caffeine intake (OR=10.82; 95% CI, 3.78–31), increased birth order (OR=6; 95% CI, 1.55–23), low calorie diet (OR=5.15; 95% CI, 1.79–14), occasional use of fruit and vegetables (OR=3.38; 95% CI, 1.67–6.82), alcohol intake (OR=3.05; 95% CI, 1.24–7.50), and, above all, lack of correct folate supplementation at any time point of pregnancy (OR=20.54; 95% CI, 5.41–77). The strength of the association with spina bifida risk of caffeine intake and increased birth order was found even stronger than in univariate analysis. Lack of folate supplementation at any time point of pregnancy remained the strongest risk factor; however, the effect was lower than in univariate analysis.

Table 2 Periconceptional and first-trimester exposures of the mothers and NTD risk

Exposures *n* (%) yes	Cases (*N*=133)	Controls (*N*=273)	OR (95% CI)	*P* value
Antibiotic use	16 (12)	20 (7.3)	1.73 (0.86–3.46)	.121
Anti-inflammatory drug use	14 (10.5)	16 (6)	1.89 (0.89–4)	.096
Antipyretic drug use	5 (3.8)	1 (0.4)	10.61 (1.23–91)	.032
Antimycotic use	2 (1.5)	3 (1.1)	1.37 (0.23–8.32)	.730
Antihistamine drug use	2 (1.5)	7 (2.6)	0.58 (0.12–2.83)	.501
FAA	2 (1.5)	0	10.1 (0.48–212)	.543
Multivitaminic supplements	8 (6)	20 (7.4)	0.81 (0.35–1.89)	.625
Iron supplements	22(16.5)	63 (23.1)	0.66 (0.40–1.13)	.130
Ovulation-stimulating drugs	4 (3)	4 (1.5)	2.08 (0.51–8.47)	.304
Diagnostic x-rays	7 (5.3)	8 (3)	1.84 (0.65–5.19)	.249
Anesthesia	1 (0.8)	4 (1.5)	0.51 (0.06–4.60)	.548
Toxic (solvents and pesticides)	5 (3.8)	1 (0.4)	10.62 (1.23–91)	.032
Living near waste sites or polluting industries	12 (14.6)	12 (4.6)	3.57 (1.54–8.29)	.003

OR odds ratio, *CI* confidence interval, *Ref.* reference group, *FAA* folic acid antagonists

Discussion

This study is the first to provide a comprehensive overview of maternal periconceptional and first-trimester lifestyle factors determining spina bifida risk in Italian women, which is a low-risk population [3]. Since this was intended as an explorative study, we simultaneously analyzed many different periconceptional factors. Overall, our data substantiate both the well-known and more recent [5, 35]

Table 3 Periconceptional and first-trimester lifestyle of the mothers and NTD risk

Variable	Cases (*N*=133; %)	Controls (*N*=273; %)	OR (95% CI)	*P* value
Smoking [a]				
No	97 (72.9)	226 (83.7)	Ref.	
Yes	36 (27.1)	44 (16.3)	1.91 (1.16–3.14)	.012
Coffee[b]				
No	26 (19.7)	99 (36.4)	Ref.	
<3 cups/day	59 (44.7)	150 (55.1)	1.50 (0.88–2.54)	
>3 cups/day	47 (35.6)	23 (8.5)	7.78 (4.02–15.05)	<.001
Alcohol[c]				
Less than half a liter	95 (71.4)	240 (90.2)	Ref.	
More than half a liter	38 (28.6)	26 (9.8)	3.69 (2.12–6.42)	<.001
Diet[d, e]				
Healthy	83 (63.4)	215 (87)		
Low calorie	25 (19.1)	14 (5.7)	4.63 (2.29–9.33)	
High calorie	23 (17.6)	18 (7.3)	3.31 (1.70–6.45)	<.001
Fruit and vegetable consumption[f,g]				
Regular	47 (48.5)	220 (81.5)	Ref	
Occasional	50 (51.5)	50 (18.5)	4.68 (2.83–7.74)	<.001
Emotional stress[h]				
Low	50 (37.6)	85 (35)	Ref.	
Moderate	33 (24.8)	125 (51.4)	0.45 (0.27–0.75)	
High	50 (37.6)	33 (13.6)	2.58 (1.47–4.52)	<.001

OR odds ratio, *CI* confidence interval, *Ref*. reference group

[a]Data were missing for 3 control women

[b]Data were missing for 1 NTD mother and 1 control mother

[c]Data were missing for 7 control mothers

[d]Data were missing for 2 NTD mothers 26 control mothers

[e]Healthy diet, 1,200–2,000 calories/day; low calorie diet, less than 1,200 calories/day; high calorie diet, more than 2,000 calories/day

[f]Data were missing for 36 NTD mothers and 3 control mothers

[g]Regular, 3 or more times/week; occasional, less than 3 times/week

[h]Data were missing for 30 control mothers

findings on folate protective effect, as the lack of folate supplementation in the recommended period is the strongest risk factor. The strength of the association is not substantially affected by potential confounders such as age, educational level, and parity (data not shown). The high level of risk that we found among case women who never use folic acid periconceptionally (OR=20.54 (5.41–77)), also in the context of the government policy and the low percentage of control women who took folate preconceptionally (22%), suggests that more efforts are necessary to increase awareness about the preventive effect of folic acid. The relatively high prevalence of control women using folic acid preconceptionally among some sociodemographic groups (82% of women with high school education, 65% of women with a medium to high family income, and 79.6% of primiparous women, data not shown) is encouraging. While some progress is being made, much more efforts remain to be done to increase folic acid consumption among women of childbearing age. Substantial knowledge improvement can be obtained carrying out information campaigns addressed to gynecologists, general practitioners, and all women of childbearing age.

Our results also emphasize that, even if with lower effects, high caffeine intake, low calorie diet, occasional consumption of fruit and vegetables, and increased birth order contributed to the risk. Low educational level, medium annual family income, maternal age, smoking habits, high calorie diet, high emotional stress, and environmental pollution were significant factors in the univariate analysis, but were not confirmed in the multivariate models. Taken together, lack of folate supplementation, high caffeine and alcohol intake, and a diet with poor content of fruit and vegetables suggest a common underlying mechanism, i.e., a disturbed folate/homocys-

Table 4 Multivariate models of the association between maternal risk factors and NTD occurrence

Risk factors	OR (95% CI)	*P* value
Folic acid supplements		
Before and after conception	Ref.	
After conception	2.38 (0.64–8.88)	
Never	20.54 (5.41–77)	0.0001
Coffee		
None	Ref.	
<3 cups/day	1.98 (0.87–4.50)	
>3 cups/day	10.82 (3.78–31)	0.0001
Fruit/vegetable consumption[a]		
Regular	Ref.	
Occasional	3.38 (1.67–6.82)	0.001
Diet[b]		
Balanced	Ref.	
Low calorie	5.15 (1.79–14)	
High calorie	1.12 (0.37–3.37)	0.01
Alcohol		
Less than half a liter	Ref.	
More than half a liter	3.05 (1.24–7.50)	.01
Birth order		
1	Ref.	
2	1.16 (0.54–2.47)	
≥3	6 (1.55–23)	.03

OR odds ratio, *CI* confidence interval, *Ref*. reference group

[a]Healthy diet, 1,200–2,000 calories/day; low calorie diet, less than 1,200 calories/day; high calorie diet, more than 2,000 calories/day

[b]Regular, 3 or more times/week; occasional, less than 3 times/week

teine metabolism. Folic acid deficiency has also been proposed as a possible cause of spina bifida in multiparous women, since blood levels decreased with an increase in parity [1]. Low folate status and impaired folate metabolism may affect homocysteine remethylation, resulting in mildly elevated levels of circulating homocysteine, a condition known as hyperhomocysteinemia. Moderately elevated homocysteine levels have been correlated with an increased risk for multifactorial diseases, including NTDs [26, 40, 45, 46]. Unfortunately, we had no information on the folate status of women at the time of pregnancy and on possible genetically determined disturbances of their folate metabolism, but future research should be focused on these aspects. Nevertheless, our data seem to confirm the hypothesis that nutritional folate deficiency and, more generally, a poor diet in the early months of pregnancy is associated with NTD offspring. [20] The fact that Italian women who did not consume folate-rich foods during the periconceptional period have an increased risk compared with users support an inverse relation between Mediterranean diet and incidence of NTD. According to the comprehensive estimate of the global burden of NTDs, Italy is a low-incidence area, and researchers believe the Mediterranean diet could be the reason [18].

The impact of periconceptional alcohol consumption on the growth and development of the fetus, referred to as fetal alcohol syndrome, is well established [16, 36]. However, the association of alcohol consumption with major congenital anomalies, particularly NTDs, remains a matter of debate. The few epidemiologic studies that have evaluated the potential association between alcohol intake during early pregnancy and NTDs concluded that such consumption did not affect the risk [27, 29, 39, 42]. Our findings of a moderately elevated risk associated with periconceptional alcohol consumption are not in agreement with the results of these studies in the definition of the periconceptional period, the methods for quantifying alcohol consumption, and the time period over which data were collected. In general, we cannot compare our results with those of previous studies, as we considered as reference group women who, in the periconceptional period, drunk less than half a liter a day of alcoholic drinks, corresponding to 480 g of alcohol, whereas previous studies simply distinguished between women who consumed alcohol and those who did not or considered the frequency of alcohol consumption, that is the number of drinks per day [27, 29, 38, 42].

In our study, multivariate analysis showed that high caffeine intake (≥300 mg/day equivalent to more than three cups per day) was associated with NTD risk. An underestimation of caffeine intake cannot be excluded since we lacked information on the daily intake of other major sources of caffeine, such as beverages, foods, and some medications. The findings of our study are in agreement with those of a recent large Californian population-based study reporting a modestly increased risk associated with total caffeine consumption and each caffeine source except caffeinated tea [42]. Caffeine is a methylxanthine that can cross the placenta during pregnancy and might act as vitamin B_6 antagonist, thus, possibly impairing the breakdown of homocysteine through the vitamin B_6-dependent transsulfuration pathway, leading to hyperhomocysteinemia [10]. This could explain the association between caffeine and NTDs, although interaction with other teratogens has inhibitory effects on DNA repair, and release of catecholamines or corticosterone also remains plausible explanation [33]. Although there is substantial evidence that caffeine is teratogenic in animals, [6, 52] so far, there is no convincing evidence that it is associated with birth defects in humans [19, 23, 34]. Further studies are needed to confirm whether women with high intake of caffeine in their periconceptional period are at increased risk for spina bifida-complicated pregnancies.

Concerning the limitations of our study, referral bias proved to be of major importance for hospital-based studies. Concerns also arise over the use of prevalent rather

than incident cases. Multicentre- or population-based studies guarantee more representative sample and contribute to eliminate bias from differential admission of cases. Unfortunately, to our knowledge, this is the first study carried out on Italian women with merely explorative purposes, and so far, data pooling does not seem feasible. The forementioned effect estimates could be also biased by recall bias. Case women may have been more likely to remember these events as they sought explanations for having a child with congenital anomalies. Studies of women comparing exposures prospectively with those measured retrospectively, however, report minimal difference in recall between women who had infants with birth defects and those who did not [47, 50]. Evidence is emerging that the role of recall bias is considered to be rather small in case–control studies focused on congenital malformation [15]. An increased risk of recurrence following a NTD-affected pregnancy has been well reported [12, 14, 53]. Out of the 133 case mothers, five (4%) reported a previous NTD-affected pregnancy (data not shown). However, we could not estimate the associated risk because inclusion criteria for control women included no history of birth defect. Finally, our findings have to be interpreted cautiously because of the small sample size.

Our study, as well as other latest papers, was characterized by its ability to examine a variety of potential covariables [4, 53], the availability of medical records for the entire case group and for the majority of the controls, the high participation rates among the study groups, the high significance of the results, and their biological plausibility. On the basis of the results of this explorative study, further studies on larger cohorts of participants based on the development of a questionnaire addressing specific questions will throw further light on the association between some maternal factors and spina bifida risk and provide information for public health strategies.

Conclusion

This study has identified some maternal periconceptional lifestyle factors associated with an increased spina bifida risk among Italian women. This association raises the hypothesis of a disturbed folate/homocysteine metabolism as a common underlying mechanism. This information can be included in future preconceptional counseling.

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