

Short Report

Folate concentrations during pregnancy and autistic traits in the offspring. The Generation R Study

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In a population-based study, we examined the associations of maternal plasma folate concentrations at 13 weeks of gestation and prenatal folic acid supplement use with autistic traits in the offspring at the age of six years. Parent-reported autistic traits were assessed using the Social Responsiveness Scale short form. Maternal folate was not associated with autistic traits in the offspring. In contrast, prenatal folic acid use was associated with less child autistic traits. Future research should focus on the timing of the potential effect of prenatal folate on the development of autistic traits in combination with clinical diagnosis of autism in the offspring.

Introduction

For decades, scientists have been trying to unravel the etiology of autism spectrum disorders (ASD). Despite advances in genetic research, the causes of ASD remain unclear.¹ As a result, research into environmental risk factors for autism has increased, with particular interest for risk factors during the prenatal period. Recently, strong evidence has been found for an association of periconceptional folic acid supplement use with reduced ASD risk in the offspring.² However, to further support a potential relationship between folic acid supplement use and ASD, nutritional biomarkers need to be examined in order to gain insight in the underlying mechanism.

In this prospective population-based study, we addressed this issue by exploring whether folate concentration in pregnancy predicted autistic traits in the offspring at six years of age. We hypothesized that higher maternal folate concentrations are associated with less autistic traits in the offspring.

Methods

Within the Generation Study, a population-based birth cohort in Rotterdam, the Netherlands³, we measured maternal plasma folate concentrations in early pregnancy (median: 13.2 weeks of gestation; 90% range: 10.5–17.2 weeks). In line with previous studies, we excluded mothers of multiple births and those of neonates with a gestational age <32 weeks at birth or a birth weight <2500 g to isolate folate exposure from other exposure reported to increase the risk of ASD.⁴ As a result, valid folate concentrations were available in 5591 mothers of single live-born neonates. Folate concentration was used as a continuous variable in the analyses (nmol/l per SD) and as a dichotomous variable, dichotomized at the level of deficiency (<7 nmol/l⁵, $n=214$). Additionally, folic acid supplementation was assessed by questionnaire early in pregnancy. In line with previous publications, folic acid supplement use was categorized in four groups: (i) preconceptional start, (ii) start within the first 10 weeks of pregnancy, (iii) start after the first 10 weeks of pregnancy and (iv) no

use (reference category).⁶ Folate concentrations in mothers who started supplementation preconceptionally were higher than in non-users (22.6 vs. 10.0 nmol/l, $t=46.6$, $P<0.001$). Information on autistic traits was available in 3893 (70%) children at the age of six years.

We assessed parent-reported autistic traits using the Social Responsiveness Scale (SRS) short form (median score: 0.17, 95% range: 0.00–0.88) and the Pervasive Developmental Problems (PDP) subscale (median score: 2.00, 95%: 0.00–8.74) of the Child Behaviour Checklist⁷ (mean age = 6.2 ± 0.5 years). SRS scores were transformed by square root and analyzed continuously. To facilitate clinical interpretation, we defined a probable autistic child using stringent criteria [PDP score >98th percentile and SRS score in the top 5% of the sample ($n=72$, 1.8%)] in line with previous studies.⁷

Multivariate linear regression was used to explore associations of both maternal folate concentrations and folic acid supplementation with children's autistic traits (SRS scores). The odds of being a probable autistic child with increase in maternal folate concentration were calculated using multivariate logistic regression. Basic models were adjusted for gestational age at venipuncture and gender and age of the child at assessment. Parental ethnicity, education, age and psychopathology during pregnancy, family income, marital status, pregnancy planning, and maternal smoking and alcohol consumption during pregnancy, parity and pre-pregnancy BMI were included as confounders in fully adjusted models. Step-wise forward regression was used to explore to which confounders the attenuation in effect could be attributed.

Missing values on folic acid supplementation (10.2%), covariates (0.2–26.8%) and child outcomes (SRS = 18.3%; PDP = 3.8%) were imputed using the Markov Chain Monte Carlo multiple imputation technique, generating five datasets. A complete overview of the population characteristics and percentages of missing values for all used variables is given in supplementary table 1. Analyses were conducted using Stata version 12.0 (StataCorp). The study was approved by the Medical Ethics Committee at Erasmus MC, Rotterdam, the Netherlands.

Results

We found that children of mothers who started using folic acid supplements before conception had lower scores on autistic traits than children whose mother did not use folic acid supplements ($B_{\text{preconceptional start vs. 'no use'}} = -0.129$, 95% CI: -0.155 to -0.102 , $P < 0.001$). This association attenuated, but remained statistically significant after adjusting for confounders (fully adjusted model: $B_{\text{preconceptional start vs. 'no use'}} = -0.042$, 95% CI: -0.068 to -0.017 , $P = 0.001$). Similar results were found for the other two groups of folic acid supplement use (fully adjusted model: $B_{\text{start < 10 weeks vs. 'no use'}} = -0.041$, 95% CI: -0.066 to -0.016 , $P = 0.001$; $B_{\text{start > 10 weeks vs. 'no use'}} = -0.057$, 95% CI: -0.089 to -0.025 , $P = 0.001$).

For maternal folate concentrations, no association with child autistic traits was found (fully adjusted model: $B = -0.004$, 95% CI: -0.013 to 0.004 , $P = 0.30$). The initial association in the basic model disappeared after joint inclusion of maternal prenatal psychopathology, maternal education and family income (table 1). Similarly, we did not find evidence for an association of extreme low maternal folate concentrations (folate deficiency) with child autistic traits (fully adjusted model: $B = 0.022$, 95% CI: -0.014 to 0.058 , $P = 0.22$). Analyses using a different cut-off (8.1 nmol/l, i.e. lowest 10% in study sample) showed similar results.

Finally, we found that the odds of being a probable autistic child did not decrease with higher maternal folate concentration (fully adjusted model: OR = 1.03, 95% CI: 0.76 to 1.39).

Discussion

In this population-based study, we did not find evidence for an association of maternal folate concentrations in early pregnancy with autistic traits in the offspring at the age of six years. Similar to earlier studies,^{2,8} we found that prenatal folic acid supplement use—although strongly confounded—was associated with less child autistic traits. Interestingly, in contrast to previous studies, we did not find this association to be specific for preconceptional start with folic acid supplementation.

Table 1 Maternal folate concentration during pregnancy and child autistic traits at six years

	Autistic traits (n = 3893) ^a	
	Model 1 ^b B (95% CI)	Model 2 ^c B (95% CI)
Folate (per SD) ^d	-0.026 (-0.035 to -0.017) ^e	-0.007 (-0.016 to 0.001) ^f
Maternal psychopathology		0.092 (0.058 to 0.127)
Maternal education		
Higher		Reference
Secondary		0.058 (0.038 to 0.078)
Primary		0.121 (0.082 to 0.160)
Family income		
>2000 €/month		Reference
1200–2000 €/month		0.035 (0.013 to 0.057)
<1200 €/month		0.077 (0.037 to 0.118)

CI = Confidence interval, SD = Standard Deviation

a: Social Responsiveness Scale, mathematically transformed (square root) to satisfy the assumption of normality in linear regression model.

b: Model 1: adjusted for gestational age at venipuncture, gender and age of the child.

c: Model 2: model 1, additionally adjusted for maternal psychopathology, education and family income.

d: Folate concentrations in nmol/l.

e: $P < 0.001$.

f: $P = 0.09$.

The association between prenatal folic acid supplementation and child autistic traits, although repeatedly reported, may be explained by residual confounding. Prenatal folic acid supplement use, a marker of good health literacy, is associated with many health-conscious behaviours that decrease the background risk of autistic traits in the offspring. An alternative explanation for our inconsistent findings between folic acid supplementation and folate concentrations might be the timing of biomarker assessment. Although we did not find an effect of folate concentrations measured at 13 weeks of gestation, the potential effect of maternal folate on the development of autistic traits in the offspring might occur earlier or later in pregnancy.

Our findings do not provide evidence for a biological pathway between prenatal folic acid supplement use and child autistic traits through higher maternal folate concentrations. Yet, our study was limited by the fact that clinical diagnoses of autism were not at our disposal. Autistic traits are defined as subclinical deficits in socialization, communication, and restricted/stereotypic behaviours that do not meet formal criteria for an ASD diagnosis.⁹ However, although not meeting strict diagnostic criteria, comorbid psychiatric traits and psychosocial difficulties have been reported in individuals with increased levels of autistic traits.¹⁰

Nevertheless, to gain further insight in the biological underpinnings of ASD, future research should focus on the timing of the potential effect of prenatal folate on the development of autistic traits in combination with clinical diagnosis of autism in the offspring.

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Conflicts of interest: None declared.

Key points

- Periconceptional folic acid supplement use has been associated with reduced risk of ASD in the offspring.
- Within a large population-based sample, we found no evidence to support a potential biological relationship via maternal folate concentrations in early pregnancy.

- Future research should focus on the timing of the potential effect of prenatal folate on the development of autistic traits, which may lead to new guidelines about continuation of folic acid supplement use in pregnancy.

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Cybervictimization and somatic and psychological symptoms among Italian middle school students

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Background: Existing literature clearly documents the association between cybervictimization and psychological symptoms; less clear is the association between cybervictimization and somatic symptoms. This study aims to verify the association between cybervictimization and both psychological and somatic symptoms on a representative sample of Italian early adolescents. **Methods:** This study used data from 24 099 students aged 13 years participating in the 2009/2010 Health Behaviour in School-aged Children Survey. Self-completed questionnaires, devised by the HBSC international group, were administered in classrooms. Multilevel models of logistic regression (controlling for traditional bullying victimization, computer use and demographics) were used to investigate the association between cybervictimization and psychological and somatic symptoms. **Results:** Overall, 3.1% of the students reported having been bullied frequently electronically and 8.7% occasionally (compared, respectively, to 4.0 and 9.2% victims of traditional forms of bullying). Overall, prevalence of students reporting psychological and somatic symptoms was 32.5 and 12.0%, respectively. Being victims of cyberbullying was positively associated to students' psychological and somatic symptoms, after controlling for traditional bullying victimization and computer use. **Conclusion:** Cybervictimization has similar psychological and somatic consequences for boys and girls, thus suggesting that intervention and prevention efforts should focus on both gender groups.

Introduction

Bullying at school is a relatively common experience among students in many countries throughout the world,¹ and Italy alike.² Recently, the proliferation of electronic communications technologies has afforded youth a new means of peer aggression, called 'cyberbullying'.^{3,4} Cyberbullying includes aggressive behaviour through e-mail, instant messaging, in a chat room, on a website or through digital messages or images sent to a cell phone.

Recent meta-analyses have documented adverse consequences of being bullied at school on youth's lives, including physical health symptoms,^{5–7} psychological problems (e.g., depression, anxiety, low self-esteem),^{8,9} and suicidal ideation.¹⁰ Comparatively much less is known about the risks of cybervictimization for youth's adjustment and well-being. To date, research has shown that, similarly to what

has been documented for traditional bullying, victims of cyberbullying report more depression, anxiety and loneliness compared to non-victimized peers, whereas somatic problems related to the experience of cybervictimization have been investigated to a limited extent.^{3,11} Therefore, there is clear need for large scale, nationally representative studies that further investigate the relationship between cybervictimization and both somatic and psychological symptoms. Moreover, it is necessary to estimate these associations after controlling for the concurrent occurrence of traditional victimization, to test whether cybervictimization uniquely contributes to the negative health outcomes. Another possible confounding variable that needs to be controlled for is Internet use, which is positively associated with both cybervictimization¹² and psychological problems;^{13,14} time spent on sedentary screen-based activities (such as computer use) is also a risk factor for adolescents' physical