Published in final edited form as:
Birth Defects Res A Clin Mol Teratol. 2011 October ; 91(10): 885–893. doi:10.1002/bdra.22848.

Lack of maternal folic acid supplementation is associated with heart defects in Down syndrome: a report from the National Down Syndrome Project

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Abstract

BACKGROUND—Maternal folic acid supplementation has been associated with a reduced risk for neural tube defects, and may be associated with a reduced risk for congenital heart defects, and other birth defects. Individuals with Down syndrome are at high risk for congenital heart defects and have been shown to have abnormal folate metabolism.

METHODS—As part of the population-based case-control National Down Syndrome Project, 1011 mothers of infants with Down syndrome reported their use of folic acid-containing supplements. These data were used to determine whether lack of periconceptional maternal folic acid supplementation is associated with congenital heart defects in Down syndrome. We used logistic regression to test the relationship between maternal folic acid supplementation and the frequency of specific heart defects correcting for maternal race/ethnicity, proband sex, maternal use of alcohol and cigarettes, and maternal age at conception.

RESULTS—Lack of maternal folic acid supplementation was more frequent among infants with Down syndrome and atrioventricular septal defects (OR=1.69; 95% CI, 1.08–2.63; *P*=0.011) or atrial septal defects (OR=1.69; 95% CI, 1.11–2.58; *P*=0.007) than among infants with Down syndrome and no heart defect. Preliminary evidence suggests that the patterns of association differ by race/ethnicity and sex of the proband. There was no statistically significant association with ventricular septal defects (OR=1.26; 95% CI, 0.85–1.87; *P*=0.124).

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

The authors have no competing interests to declare.

CONCLUSIONS—Our results suggest that lack of maternal folic acid supplementation is associated with septal defects in infants with Down syndrome.

Keywords

Atrial septal defect; Atrioventricular septal defect; Congenital heart defect; Down syndrome; Folic acid

INTRODUCTION

Individuals with Down syndrome (DS), the clinical consequence of trisomy 21, exhibit a wide range of phenotypes. Congenital heart defects (CHD) occur in approximately 40% of DS cases and range from small atrial septal defects (ASD) or ventricular septal defects (VSD) to complete atrioventricular septal defects (AVSD) and other serious heart defects, such as tetralogy of Fallot (TOF) (e.g. (Freeman et al., 2008; Freeman et al., 1998). We recently reported that the frequencies of AVSD and secundum ASD (ASD II), but not VSD, in the DS population vary by sex and race/ethnicity of the proband, suggesting underlying genetic or race/ethnicity-specific environmental risk factors (Freeman et al., 2008). Compared with non-Hispanic white infants with DS, non-Hispanic black infants have double the risk and Hispanic infants have half the risk for an AVSD. Also, there is a nearly 2-fold excess of female probands among DS cases with an AVSD, despite a male-to-female ratio of 1.15 among all infants with DS (Freeman et al., 2008; Kallen et al., 1996). An excess of female nonsyndromic AVSD cases has also been noted (Ferencz et al., 1997).

Folate, a vital nutrient, donates methyl groups for purine and pyrimidine synthesis, methylation of DNA and proteins, and conversion of homocysteine to methionine. DNA methylation is used for critical cellular functions such as imprinting, X-chromosome inactivation, and long term gene silencing (Bernstein et al., 2007). The importance of the folate pathway in development is clear from the association between maternal folic acid intake and neural tube defects (NTDs) (1991; Czeizel and Dudas, 1992). In 1992 the US Public Health Service (USPHS) issued a recommendation that all fertile women consume 0.4 mg of folic acid per day to reduce the risk of NTDs in offspring (CDC, 1992). Studies of maternal folic acid supplementation in the etiology of CHD (Bailey and Berry, 2005; Botto et al., 1996; Botto et al., 2004; Scanlon et al., 1998; Shaw et al., 1995; van Beynum et al., 2010) and other birth defects (Bailey and Berry, 2005; Botto et al., 2004) suggest a role for the folate pathway, although significant associations have not been seen in all studies.

The many functions of the folate pathway are mediated by enzymatic processes. Several studies have found that polymorphisms in folate pathway genes like *5,10-methylenetetrahydrofolate reductase (MTHFR), 5-methyltetrahydrofolate-homocysteine methyltransferase* (*MTR*), and *5-methyltetrahydrofolate-homocysteine methyltransferase reductase* (*MTRR*) reduce the enzyme activity of their gene products (Frosst et al., 1995; Harmon et al., 1999; van der Put et al., 1997; Weisberg et al., 1998). Polymorphisms in these pathway genes and others, such as the chromosome 21-linked reduced folate carrier gene (*SLC19A1*), have been associated with CHD in some, but not all studies (Goldmuntz et al., 2008; Hobbs et al., 2006; Locke et al., 2010; McBride et al., 2004; Mitchell et al., 2010; Pei et al., 2006; Shaw et al., 2005; Shaw et al., 2003; van Beynum et al., 2006).

In addition to studies of folate pathway gene polymorphisms in nonsyndromic CHD, the biochemical consequences of trisomy 21 led us to consider the folate pathway to explain the increased risk for CHD among infants with DS. Enzymatic and biochemical evidence suggests that individuals with DS have abnormal folate/homocysteine metabolism. Overexpression of the *cystathionine beta synthase* (*CBS*) gene, located on chromosome 21,

creates a functional folate deficiency in tissues with trisomy 21 (Chadefaux et al., 1985). Folate pathway components such as homocysteine, methionine, S-adenosyl methionine and S-adenosylhomocysteine are reduced in individuals with DS (Pogribna et al., 2001). In support of this hypothesis we recently showed an associated between polymorphisms in *SLC19A1* and AVSD in DS (Locke et al., 2010). In the current study we use the large population-based epidemiological dataset collected through the National Down Syndrome Project (NDSP) to test the hypothesis that maternal folic acid supplementation prior to fetal heart development is associated with CHD in DS.

MATERIALS AND METHODS

Population ascertainment

Based at Emory University in Atlanta, Georgia, the NDSP enrolled families of infants with DS born from 2001 through 2004 at six sites across the country. Each site was linked to a birth defects surveillance system. We previously reported the details of ascertainment and recruitment (Freeman et al., 2007). All NDSP sites obtained institutional review board approvals and informed consent from participants.

The NDSP included live-born infants with standard trisomy 21 or mosaic trisomy 21 born during the study period to English- or Spanish-speaking mothers. Infants with DS due to a translocation were excluded as were families whose infants died after birth and before study enrollment. Those excluded because the infant died before enrollment represented less than 5% of identified cases and did not differ proportionally in maternal race/ethnicity or proband sex from those who were liveborn. For the current study, we have further excluded infants with mosaic trisomy 21 and those with both trisomy 21 and another clinically relevant chromosome abnormality.

Race and ethnicity of the mother was determined by the mother's self-report. Methods for the collection and abstraction of medical records documenting CHD and other birth defects were previously described (Freeman et al., 2008). Briefly, each recruitment site abstracted infant medical records and entered the information onto a structured clinical form, which was reviewed by a single clinically trained individual. A pediatric cardiologist was consulted as necessary. Each occurrence of a specific type of CHD was counted. For example, infants with more than one heart defect were included as cases in each relevant group. Complex heart defects (e.g. complete AVSD, TOF, etc.) were counted as single defects. Only those clearly described as ASD II were counted as ASD II. Control infants were those with a structurally normal heart, patent foramen ovale (PFO), and/or patent ductus arteriosus (PDA). The use of echocardiography to document normal heart status at five out of six recruitment sites was over 90% and at the sixth site (selected geographic area in California) was over 70% among probands with DS. In the remaining cases, physical exam was used (Freeman et al., 2008).

Determination of maternal behavior and exposures

Participating mothers completed questionnaires administered by trained study personnel at the time of enrollment in the NDSP (Freeman et al., 2007). Using data from these questionnaires, we determined maternal use of folic acid-containing supplements, alcohol, and cigarettes, as well as education. Mothers were asked about prenatal vitamin, vitamin, and supplement intake for three periods: before pregnancy, the first three months of pregnancy, and after the first three months of pregnancy. We assigned mothers to "supplemented," "non-supplemented," "uncertain," or "missing" folic acid use groups. Human heart development occurs between the fourth and eighth weeks of pregnancy (calculated from the last menstrual period (Sadler, 2005). Mothers who were taking a folic

acid-containing vitamin or supplement before becoming pregnant and those who began taking a folic acid-containing supplement within the first four weeks of pregnancy were assigned to the "supplemented" group. Those who began a folic acid-containing supplement during or after the eighth week of pregnancy and those who took no folic acid-containing supplement were assigned to the "non-supplemented" group. Although formation of the cardiac septa begins during the sixth week of pregnancy, we conservatively excluded those with "uncertain" supplementation (those whose folic acid supplementation started between the fourth and eighth week of pregnancy). Those with missing data were excluded from the analysis. Maternal education was determined to be either less than or at least a high school education. Mothers were asked about alcohol and cigarette use during two time periods: the first month and the second through third months of pregnancy. We used information about use during the first month instead of the second through third months of pregnancy, since the exposure was higher (Table 2). For alcohol use, those who reported consuming at least one alcoholic drink per week during the time period were considered to have used alcohol. For cigarette use, those who smoked at least one cigarette per week during the time period were considered to have smoked.

Statistical analysis

We used chi-square analysis in comparisons of frequency distributions between case groups. For each CHD, we used logistic regression analysis adjusting for maternal race/ethnicity and proband sex to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association of CHD with folic acid supplementation using infants with DS without CHD as controls. Maternal age at birth of the infant, maternal education, alcohol use, and smoking did not contribute significantly in the AVSD or VSD model (i.e., step-wise removal of each did not change the OR by > 10%) and were removed. Maternal smoking (at least one cigarette per day) in the first month of pregnancy contributed significantly to the ASD II model and was included in all ASD II models except Hispanics alone due to the small number of Hispanic mothers who smoked (n=7/390). In addition, none of the interaction terms with folic acid use and the primary covariates were significant and none contributed significantly to the model as determined by the log likelihood method. Irrespective of statistical significance of the interaction terms, we stratified each analysis by factors previously known to be associated with AVSD; namely proband sex and race/ethnicity. Statistical analysis was performed using Statistical Analysis Software (SAS; SAS Institute Inc., Cary, NC).

Our primary hypothesis that lack of folic acid supplementation increases the risk for CHD is unidirectional. Thus we provide *P*-values for CIs around the ORs for lack of folic acid supplementation reflecting a one-sided test at a significance level of 0.05. Applying a Bonferroni correction for the three hypotheses originally tested (the association of lack of maternal folic acid use and AVSD, ASD II, or VSD in the proband) adjusts the significance level to $P=0.017$. No correction was applied to the post-hoc stratified analyses.

RESULTS

The study population

The NDSP identified 1469 infants with DS and, of those families, 1079 (73.5%) participated by completing a maternal questionnaire and providing access to medical records. There was no difference in the frequency of CHD, maternal race/ethnicity, or proband sex ratio between eligible infants and enrolled infants (Table 1).

The study population consisted of 510 non-Hispanic white mothers (referred to as white), 111 non-Hispanic black mothers (referred to as black), and 390 white Hispanic mothers for a

total of 1011. There were too few black Hispanic mothers (n=9) and Asian mothers (n=37) to be included in further analysis. The demographics and frequencies of maternal behaviors for each type of CHD are provided in Table 2.

Among infants with an AVSD, there were more black and fewer Hispanic mothers than white mothers (Table 2). These differences reflect an increased OR for AVSD for blacks compared with whites of 1.86 (95% CI, 1.17–2.96, *P*=.009) and a decreased OR for AVSD for Hispanics compared with whites of 0.51 (95% CI, 0.34–0.76, *P*<0.001), adjusting for proband sex. The racial/ethnic distribution among mothers of infants with an ASD II differed from those of infants with no ASD II (Table 2): there were more black (OR=2.00; 95% CI, 1.23–3.24, *P*=0.005) and more Hispanic (OR=1.44; 95% CI, 1.02–2.03, *P*=0.04) mothers compared with white mothers, adjusting for proband sex. With respect to proband sex, there were more females compared with males among those with an AVSD compared with those without an AVSD (Table 2), leading to an OR of 2.08 (95% CI 1.48–2.94, *P*<0.0001), adjusted for race/ethnicity. The proband sex ratio did not differ significantly for infants with an ASD II (95% CI 1.29, 0.94–1.77, *P*=0.12) or a VSD (Table 2). These patterns were consistent with those previously reported for all eligible infants (Freeman et al., 2008). Given these differences in association between AVSD, ASD II, and VSD with maternal race/ethnicity and proband sex, as well as, the previously reported female bias between syndromic and nonsyndromic AVSD, these CHDs were analyzed separately. There were insufficient cases of TOF or other CHD to be included in this or subsequent analyses.

Maternal supplement use and CHDs in DS

We next investigated the relationship between maternal use of folic acid-containing supplements and the frequency of CHD in DS probands adjusting for both race/ethnicity and sex. The proportion of white, black, and Hispanic mothers in the “uncertain” (21%, 18%, and 18%, respectively) and “missing” (2%, 1%, and 2%, respectively) groups did not differ (*P*=0.77). A total of 407 mothers of infants in the “supplemented” group (white: n=286, black: n=35, Hispanic: n=86) were compared with 392 mothers of infants in the “non-supplemented” group (white: n=109, black: n=55, Hispanic: n=228). Among these 799 mothers, a higher proportion of white mothers than black or Hispanic mothers used folic acid-containing supplements (72%, 39%, and 27%, respectively; p<0.0001).

AVSD—Logistic regression analysis regressing AVSD case-control status (reference: DS proband with no CHD, controlling for proband sex and maternal race/ethnicity) against folic acid use as the primary exposure demonstrated a statistically significant association between AVSD in probands with DS and mothers who did not take a folic acid-containing supplement (OR=1.69; 95% CI 1.08–2.63; *P*=0.011, Table 3). Hispanic maternal ethnicity and proband sex were significant in this model (Table 3). When folic acid use by race/ethnicity or by proband sex interaction terms were added to the model, they were not significant. Irrespective, we stratified by these factors in a post-hoc analysis. Among the three ethnic/racial groups, the association between lack of folic acid supplementation and AVSD reached significance only among Hispanic mothers (OR=3.45; 95% CI, 1.36–8.71; *P*=0.014, Table 4). In the stratified analysis by proband sex, there was a statistically significant association between lack of folic acid use and AVSD (OR=2.32; 95%CI 1.28–4.20; *P*=0.010) among males adjusting for race/ethnicity. For females, there was no statistically significant association (OR=1.37; 95% CI, 0.84–2.24; *P*=0.146). We observed a correspondingly lower male:female sex ratio among infants with an AVSD born to supplemented (M:F=0.46) versus non-supplemented mothers (M:F=0.86). We had insufficient cases to stratify the analysis by both race/ethnicity and sex of the proband.

ASD II—Using the same logistic regression approach, we examined the influence of folic acid-containing supplements among DS cases with an ASD II compared with DS controls with no CHD. Controlling for proband sex, maternal race/ethnicity, and maternal smoking demonstrated a significantly increased OR for lack of folic acid use, 1.69 (95% CI, 1.11–2.58; *P*=0.007, Table 3). Interaction terms with folic acid use by race/ethnicity or by proband sex were not significant. Again, as a post-hoc analysis, when stratified by race/ethnicity this observation was significant only among Hispanic mothers (OR=2.79; 95% CI, 1.32–5.87; *P*=0.004, Table 4). When stratified by proband sex, the OR for lack of folic acid use was statistically significant among females (OR=2.03; 95% CI, 1.11–3.72; *P*=0.011) but not males (OR=1.48; 95% CI, 0.81–2.70; *P*=0.106), controlling for race/ethnicity.

VSD—We found no statistical evidence for an association between folic acid-containing supplementation and VSD among all individuals with DS (Table 3) or when we stratified by race/ethnicity or sex of the proband (Table 4).

DISCUSSION

The high frequency of CHD is a significant cause of morbidity and mortality in DS.(Ballweg et al., 2007; Frid et al., 2004; Shin et al., 2007) According to a 2005 report from CDC's National Center for Health Statistics, the birth rate among women over the age of 35 has increased steadily since 1980 (Martin et al., 2007). As increasing maternal age is strongly associated with increased risk for DS, understanding and preventing its associated birth defects is of great importance. Here we demonstrate that lack of maternal folic acid supplementation is associated with an approximately 1.7-fold increased frequency of AVSD and of ASD II in DS, but no statistically significant increased frequency of VSD (Table 3). Because our data represent a diverse population, we were able to explore the relationship between maternal folic acid supplementation and DS-associated CHD in the context of race/ethnicity and proband sex.

Folic acid and proband sex

We previously observed a 2-fold increased risk for an AVSD and 1.3-fold increased risk for an ASD II among live-born DS females compared with males (Freeman et al., 2008). At the time, we suggested that the slight albeit statistically significant increased risk for an ASD II among females may be due to misclassification of AVSD cases: thus our *a priori* hypothesis focused on AVSD.

Although the folic acid use by sex interaction term was not statistically significant for any CHD, we decided to stratify the data by proband sex because of the different patterns we observed among the three major CHD groups. For AVSD, the association of lack of maternal folic acid supplementation was significant among male probands (OR=2.32; 95%CI 1.28–4.20; *P*=0.010), but not female probands (OR=1.37; 95% CI, 0.84–2.24; *P*=0.146). Among ASD II, a statistically significant association was observed among females (OR=2.03; 95% CI, 1.11–3.72; *P*=0.011) but not males (OR 1.48; 95% CI, 0.81–2.70; *P*=0.106). No difference in OR was observed for VSD by proband sex (Table 4). At this point, it is unclear whether these patterns are biologically significant or simply random effects.

The possibility of a sex-specific association between CHD and maternal folic acid use should be considered based on observations of a folic acid-related developmental disorder in which the frequency differs by sex. NTDs, in particular anencephaly, were observed more frequently in female than in male fetuses (Martinez Frias et al., 1986; Seller, 1986). Intriguingly, as the prevalence of anencephaly has declined over time, a steeper decline in female frequency has narrowed this sex ratio difference (Besser et al., 2007; Canfield et al.,

2009). These results suggest that folic acid supplementation was sufficient to reduce the risk of anencephaly in both the higher risk female fetuses as well as male fetuses. The effectiveness of maternal folic acid supplementation in reducing female NTD risk differs from the trend of our AVSD in DS data. We did find that the non-significant pattern of our ASD II associations with maternal folic acid supplementation by sex follows the same pattern that observed among NTDs. These findings may suggest a difference in tissue-specific or sex-specific thresholds of folic acid effect on heart development.

The potential for a true difference in sex-specific patterns of AVSD and ASD II associated with maternal folic acid supplementation is intriguing since this would suggest a different etiology, rather than misclassification as we originally proposed (Freeman et al., 2008). The sex-specific AVSD and ASD II patterns must be replicated. For VSD, we have not observed a sex-specific influence of folic acid use in DS, an observation which also must be replicated.

Folic acid and race/ethnicity

In this study, we observed that the use of maternal folic acid-containing supplements varied by race/ethnicity. Among the 1011 mothers included here, folic acid supplementation before the fourth week of pregnancy was lower in black and Hispanic mothers compared with white mothers (32% and 22% versus 56%). Our findings are consistent with data from the National Health and Nutrition Examination Surveys (NHANES; (Yang et al., 2007)) indicating that among non-pregnant women a smaller percentage of Hispanic and black women compared with white women consumed a minimum of 0.4 mg of folic acid per day as recommended by the U.S. Public Health Service (USPHS; (CDC, 1992))..Although serum and RBC folate levels have improved since mandatory fortification, persistent low RBC folate levels and lower reported folic acid intake have been reported in non-Hispanic blacks (Ganji and Kafai, 2006; Kant and Graubard, 2007).

In our current study, infants born to Hispanic mothers showed the most pronounced difference in AVSD and ASD II risk associated with maternal folic acid supplementation (Table 4), despite having the lowest overall risk for AVSD compared with whites and blacks and a comparable risk for ASD II compared with blacks (Freeman et al., 2008). We hypothesize that this suggests a greater impact of folic acid supplementation on the Hispanic population. Interestingly, population-based studies have demonstrated an increased risk of NTD-affected pregnancies among Hispanic women compared with white women (Canfield et al., 2009; Carmichael et al., 2008; Velie et al., 2006; Williams et al., 2005). The risk of NTD-affected pregnancies was highest among foreign-born Hispanic mothers, suggesting environmental influences contribute to NTD risk in this population (Carmichael et al., 2008; Velie et al., 2006). In our study, the majority of Hispanic mothers (83%) were born outside of the US. It would be interesting to see if the association between lack of maternal folic acid supplementation and AVSD and ASD II in this population differs in a second- or third-generation Hispanic-American population.

In addition, we suggest that the absence of a skewed sex ratio in our original report of Hispanic AVSD cases (Freeman et al., 2008) is consistent with comparable frequencies of AVSD and ASD II in males and females with DS whose mothers did not take a folic acid-containing supplement. As the majority of Hispanic mothers did not take a folic acid-containing supplement, there was no overall paucity of male AVSD cases. Among the small number of Hispanic male probands whose mothers took a folic acid-containing supplement, 0/42 (0%) had an AVSD compared with 14/126 (11%) whose mothers did not take a folic acid-containing supplement. Thus, the overall frequency of AVSD in Hispanic probands with DS was only 10%, similar to other reports (de Rubens Figueroa et al., 2003; Vida et al., 2005) suggesting a lower inherent risk for AVSD in the Hispanic population.

Strengths and weaknesses of this study

In our study, both DS case and control infants were drawn from a liveborn population. Families whose infants were stillborn or died shortly after birth were not recruited (Freeman et al., 2007). By excluding early infant deaths, infants with more severe birth defects, including some heart defects, may have been excluded. However, since the number excluded due to infant death was small (less than 5% of those identified) and since this group did not differ proportionally in maternal race/ethnicity or proband sex, this exclusion is not likely to significantly impact our findings. However, it is important to keep in mind that up to 80% of conceptions with DS are lost before birth (Hassold and Jacobs, 1984), resulting in the liveborn population being highly selected. In addition, we have no data on the number of DS conceptions electively terminated and how this group varies by race/ethnicity. An effect of maternal folic acid use on fetuses with trisomy 21 that do not survive gestation cannot be addressed in this study.

Our results differ from that of Meijer et al.(2006) who used a similar study design based on liveborn infants with DS and CHD (case) and DS and no CHD (control). They found no statistically significant association between use of folic acid-containing supplements and CHD in DS. Their study included primarily white mothers whereas our study included a more racially/ethnically diverse sample. Also the ascertainment period differed: Meijer *et al*. identified probands prior to the 1998 mandate for dietary folic acid fortification (1978–1997) whereas our study sample was ascertained after that mandate (2001–2004). In both studies, mothers were interviewed using a standardized questionnaire and cases were diagnosed using hospital records that allowed classification of CHD. Both studies paid particular attention to defining the use of folic acid supplementation during the time of heart development, although there were differences in inclusion and exclusion based on that definition. Thus, the strengths of both studies were similar. Variability of folic acid exposure (mothers in our study had exposure to folic acid through both fortification and supplementation), racial/ethnic differences, and small sample sizes once subtypes of CHD were studied may all contribute to these conflicting findings. We strongly suggest that stratification of CHD subtypes is important given differences in developmental mechanisms. Indeed, we have shown that lack of maternal folic acid supplementation was statistically associated with specific DS-associated CHD, namely AVSD and ASD II. This observation underscores the importance of ensuring phenotypic homogeneity within a study population and the utility of studying a “sensitized” population.

Despite the large sample size, the number of DS probands with the specific types of CHD was relatively modest, particularly among infants of black or Hispanic mothers. The size of this sample was insufficient to determine the impact of genetic and environmental factors known to influence folate pathway function, such as the *MTHFR* c.677C>T and c.1298A>C, *MTR* c.2756C>G, and *MTRR* c.66A>G gene polymorphisms, which are common and affect enzyme activity (Frosst et al., 1995; Harmon et al., 1999; van der Put et al., 1997; Weisberg et al., 1998). We recently reported an association between *SLC19A1* gene variants and AVSD in probands with DS (Locke et al., 2010). Brandalize *et al*. observed a higher rate of CHD in probands with DS whose mothers had a CT or TT *MTHFR* c.677 genotype when the mother did not take a folic acid containing supplement (2009). These studies suggest that maternal and proband gene-environment interactions should be explored further. Maternal use of cigarettes was inversely associated with ASD II; however, the number of mothers exposed was small and none were Hispanic. Maternal alcohol use was not associated with AVSD, ASD II, or VSD in our study. Our study did not explore maternal diet; therefore, we were unable to account for a potentially significant source of variability in folic acid consumption. Continued ascertainment of a racially/ethnically diverse population is key to further understanding the role that maternal folic acid supplementation plays in AVSD and

ASD II, and potentially VSD, risks. Moreover, confirming the differential sex-specific patterns of risks will give us insight into the etiology of these different CHD.

Future Directions

From these studies we are unable to determine if the associations observed are causal, nevertheless, the associations are significant and provide a basis for future studies. Despite a growing body of evidence that folic acid supplementation reduces not only the risk of NTDs, but also of non-syndromic CHD and other birth defects, the majority of women in this study did not take a folic acid-containing supplement prior to the fourth week of pregnancy. If maternal age in the US continues to increase, more pregnancies will be at risk for DS. Although we have found that folic acid supplementation was associated with fewer DS-associated CHD, we have not explained the differences in frequency of AVSD and ASD II among racial/ethnic groups. More studies to identify other environmental and genetic risk factors for CHD will help clinicians educate their patients on the best preventative measures prior to pregnancy.

Acknowledgments

This work was supported by NIH R01 HD38979, NIH P01HD24605, F32 HD046337, NIH RO1 HL083300, and by the technical assistance of the General Clinical Research Center at Emory University (NIH/NCRR M01 RR00039). The authors would like to thank Rupa Masse, Maneesha Yadav-Shaw, and Weiya He for excellent technical assistance, Helen Smith and Elizabeth Sablón for family recruitment and Michele Marcus for helpful discussion. In addition, we are grateful to all the personnel at each NDSP site. We thank the many families nationwide whose participation has made this study possible.

This work was funded by the National Institutes of Health (NIH).

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Table 1

National Down Syndrome Project: A comparison of the frequency of heart defects, sex ratios, and maternal race/ethnicity between all eligible and enrolled infants with DS.

	All eligible cases (Freeman et al., 2008)		**All eligible cases with maternal questionnaire (Current study)**		
	n	%	n	%	*P*-value
Heart Defect					
Atrioventricular septal defect (AVSD)	252	17.2%	178	16.5%	0.66
Atrial septal defect (ASD II)[a]	273	18.6%	203	18.8%	0.88
Ventricular septal defect (VSD)[b]	282	19.2%	213	19.7%	0.73
Tetralogy of Fallot (TOF)	39	2.7%	26	2.4%	0.70
Other	19[c]	1.3%	17[d]	1.6%	0.55
Heart Defect Summary					
Cases with ≥ one heart defect	649	44.2%	483	44.8%	0.77
Cases with no heart	820	55.8%	596	55.2%	
Total with heart defect information	**1469**		**1079**		
Sex of Proband					
Female	682	46.4%	516	48%	0.49
Male	787	53.6%	563	52%	
Total	**1469**		**1079**		
Race/Ethnicity of Mother					
White	624	43.4%	510	48.2%	
Black	183	12.7%	111	10.5%	
Hispanic	569	39.5%	399[e]	38.0%	
Asian	63	4.4%	37	3.5%	0.06
Total	**1439**		**1057**		

[a] Secundum ASD II. Excludes PFO and PFO versus ASD II.

[b] Excludes VSD that is part of an AVSD or TOF.

[c] Includes double outlet right ventricle (n=6), coarctation of aorta (n=6), dextrocardia (n=2), right aortic arch (n=5).

[d] Includes: double outlet right ventricle (n=6), coarctation of aorta (n=6), dextrocardia (n=2), right aortic arch (n=3).

[e] 390 white Hispanics, 9 black Hispanics.

Table 2

The frequency of heart defects in probands with DS stratified by maternal demographics and exposures and proband sex.

	No CHD n (%)	AVSD n (%)	ASD II n (%)	VSD n (%)
Race/Ethnicity				
White	282 (51%)	96 (56%)	79 (42%)	92 (45%)
Black	49 (9%)	34 (20%)	30 (16%)	24 (12%)
Hispanic	221 (40%)	41 (24%)	81 (43%)	89 (43%)
Proband Sex				
Female	253 (46%)	109 (64%)	102 (54%)	93 (45%)
Male	299 (54%)	62 (36%)	88 (46%)	112 (55%)
Supplement Use				
Missing	9 (2%)	4 (2%)	1 (1%)	3 (1%)
Uncertain	100 (18%)	42 (25%)	37 (19%)	36 (18%)
Suppl.	239 (43%)	60 (35%)	64 (34%)	80 (40%)
No Suppl.	204 (37%)	65 (38%)	88 (46%)	86 (22%)
Maternal Age				
<35 years	282 (51%)	97 (58%)	97 (51%)	117 (57%)
≥35 years	270 (49%)	74 (43%)	93 (49%)	88 (43%)
Maternal Education				
< high school	113 (20%)	21 (12%)	47 (25%)	52 (25%)
≥ high school	439 (80%)	150 (88%)	143 (75%)	153 (75%)
Alcohol (1st month)				
Missing	7 (1%)	0 (0%)	3 (1%)	2 (1%)
≥ 1 per wk	43 (8%)	13 (8%)	7 (4%)	15 (7%)
< 1 per wk	502 (91%)	158 (92%)	180 (95%)	188 (92%)
Alcohol (2nd/3rdmonth)				
Missing	2 (<1%)	0 (0%)	1 (<1%)	2 (1%)
≥ 1 per wk	7 (1%)	5 (3%)	4 (2%)	4 (2%)
< 1 per wk	543 (98%)	166 (97%)	185 (97%)	199 (97%)
Smoking (1st month)				
Missing	4 (<1%)	0 (0%)	2 (1%)	2 (1%)
Yes	60 (11%)	19 (11%)	9 (5%)	15 (7%)
No	488 (88%)	152 (89%)	179 (94%)	188 (92%)
Smoking (2nd/3rdmonth)				
Missing	6 (1%)	0 (0%)	1 (1%)	2 (1%)
Yes	42 (8%)	15 (9%)	7 (4%)	14 (7%)
No	504 (91%)	156 (91%)	182 (95%)	189 (92%)
Total	**552**	**171**	**190**	**205**

Table 3

Results from logistic regression models.

		No CHD	AVSD	OR (95% CI)	*P*[a]	ASD II	OR (95% CI)	*P*	VSD	OR (95% CI)	*P*
Folic acid suppl.	+ Suppl.	239 (54%)	60 (48%)	Ref.		64 (42%)	Ref.		80 (48%)	Ref.	
	- Suppl.	204 (46%)	65 (52%)	**1.69 (1.08–2.63)**	**0.011**[b]	88 (58%)	**1.69 (1.11, 2.58)**	**0.007**[b]	86 (52%)	1.26 (0.85, 1.87)	0.124[b]
Race/ ethnicity	White	218 (49%)	70 (56%)	Ref.		64 (42%)	Ref.		77 (46%)	Ref.	
	Black	42 (9%)	25 (20%)	1.58 (0.88–2.86)	0.126	24 (16%)	1.67 (0.92–3.05)	0.093	20 (12%)	1.24 (0.68–0.2.29)	0.484
	Hispanic	183 (41%)	30 (24%)	**0.41 (0.24–0.68)**	0.0006	64 (42%)	0.88 (0.56–1.39)	0.596	69 (42%)	0.97 (0.64–1.46)	0.875
Proband sex	Male	246 (56%)	49 (39%)	Ref.		73 (48%)	Ref.		90 (54%)	Ref.	
	Female	197 (44%)	76 (61%)	**2.02 (1.34–3.06)**	0.0009	79 (52%)	1.38 (0.95–2.02)	0.094	76 (46%)	1.07 (0.75–1.54)	0.706
Smoking	No	399 (90%)	-	-	-	144 (95%)	Ref.		-	-	-
	Yes	40 (9%)	-	-	-	6 (4%)	0.35 (0.14–0.86)	0.023	-	-	-
	Missing	4 (1%)	-	-	-	2 (1%)	-		-	-	-

Suppl., supplement; Ref., referent group

[a] Applying a Bonferroni correction for the three hypothesis originally tested adjusts the significance level to *P*=0.017 (see Methods).

[b] One-sided *P*-values are provided for the folic acid term

Table 4

Results from stratified logistic regression models.

	Folic acid suppl.	No CHD	AVSD	OR (95% CI)	*P*[a]	ASD II	OR (95% CI)	*P*	VSD	OR (95% CI)	*P*
Race/ethnicity											
White	+ Suppl.	161 (74%)	47 (67%)	Ref.		45 (70%)	Ref.		56 (73%)	Ref.	
	− Suppl.	57 (26%)	23 (33%)	1.39 (0.84, 2.28)	0.139	19 (30%)	1.21 (0.64, 2.29)	0.275	21 (27%)	1.06 (0.59, 1.90)	0.426
Black	+ Suppl.	17 (40%)	9 (36%)	Ref.		9 (38%)	Ref		6 (30%)	Ref	
	− Suppl.	25 (60%)	16 (64%)	1.49 (0.60, 3.70)	0.238	15 (62%)	1.72 (0.54, 5.50)	0.179	14 (70%)	1.71 (0.53, 5.50)	0.183
Hispanic	+ Suppl.	61 (33%)	4 (13%)	Ref.		10 (16%)	Ref		18 (26%)	Ref.	
	− Suppl.	122 (67%)	26 (87%)	**3.45 (1.36, 8.71)**	**0.014**	54 (84%)	**2.79 (1.32, 5.87)**	**0.004**	51 (74%)	1.41 (0.76, 2.63)	0.138
Sex											
Male	+ Suppl.	128 (52%)	19 (39%)	Ref.		31 (42%)	Ref.		41 (46%)	Ref.	
	− Suppl.	118 (48%)	30 (61%)	**2.32 (1.28, 4.20)**	**0.010**	42 (58%)	1.48 (0.81, 2.70)	0.106	49 (54%)	1.27 (0.73, 2.19)	0.199
Female	+ Suppl.	111 (56%)	41 (53%)	Ref.		33 (42%)	Ref.		39 (51%)	Ref.	
	− Suppl.	86 (44%)	35 (46%)	1.37 (0.84–2.24)	0.146	46 (58%)	**2.03 (1.11, 3.72)**	**0.011**	37 (49%)	1.26 (0.71, 2.22)	0.215

Suppl., supplement; Ref., referent group

[a]One-sided *P*-values are provided for these models