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Maternal Folic Acid Supplementation and Congenital Heart Defects in Down Syndrome

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Master of Public Health

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M.B.A., Kent State University, 2015
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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2017

Abstract

Maternal Folic Acid Supplementation and Congenital Heart Defects in Down Syndrome

By Alexander Evans

Background: Maternal folic acid supplementation may be associated with a decreased risk for some congenital heart defects. Individuals with Down syndrome have an increased risk for congenital heart defects and preliminary evidence suggests a potential mechanism for folic acid.

Methods: Mothers of a child with Down syndrome (proband) participating in the National Down Syndrome Project and Emory Down Syndrome Study reported periconceptual exposures, including use of prenatal vitamin and supplements containing folic acid. Logistic regression was used on this data to assess the relationship between maternal folic acid exposure and specific congenital heart defects while controlling for maternal race/ethnicity, proband sex, maternal use of alcohol and cigarettes, gestational diabetes, and maternal age at birth of proband.

Results: Folic acid supplementation was less frequent among probands with complete atrioventricular septal defects (OR = 0.68; $p = 0.0195$), atrial septal defects (OR = 0.64; $p = 0.0431$) and both atrial septal and ventricular septal defects (OR = 0.50; $p = 0.0179$) compared to probands with no heart defect. There was no statistically significant association with folic acid and partial atrioventricular septal defects (OR = 0.89.; $p = 0.3711$) or ventricular septal defects (OR = 0.69; $p = 0.0652$), although the similar reduction in use was estimated.

Conclusions: Our findings suggest an association of periconceptual maternal folic acid exposure and certain congenital heart defects in infants with Down syndrome.

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ACKNOWLEDGEMENTS

I would like to sincerely thank Dr. Stephanie Sherman—my thesis advisor—for her direction, patience and time throughout this process. I would also like to thank Dr. Emily Allen and Dr. Tracie Rosser for their technical support and important conversations.

I would like to thank all those involved with the National Down Syndrome Project and the Emory Down Syndrome Study, whose hard work and dedication to those studies made this thesis possible.

I would like to thank my parents—Mark and Debby Evans—for their continued love and support, and for keeping the lights on.

BACKGROUND

Congenital heart defects (CHDs) occur in up to 50% of infants born with Down syndrome (DS), with atrioventricular septal defects (AVSD), ventricular septal defects (VSD) and atrial septal defects (ASD) being the most common (Bergström et al., 2016). Freeman et al. (2008) reported differences in AVSD and ASD frequency by an infant's race/ethnicity and sex, but found no such associations for those with VSD in spite of having the same sample size. In that study, infants with DS (probands) whose mothers reported as non-Hispanic black had twice the risk for an AVSD compared to non-Hispanic white probands; Hispanic probands half the risk compared to non-Hispanic whites. Additionally, female probands had twice the risk for an AVSD compared to males. Little is known about the etiology of CHDs in DS or the differences in frequency across racial/ethnic and sex groups.

In 1992, the U.S. Public Health Service recommended 400 μg of folic acid (FA) per day for women of reproductive age to prevent neural tube defects; and in 1998, the U.S. Food and Drug Administration mandated enriched cereal grain products sold in the United States be fortified with 140 μg FA per 100 grams (Centers for Disease Control and Prevention, 2010). Studies before and after fortification have suggested a possible association between maternal FA supplementation and CHDs in both the general and DS populations, but data are conflicting (e.g., Bailey and Berry, 2005; Meijer et al., 2006; Bean et al., 2011). Bailey and Berry (2005) reviewed observational studies and concluded that most support the conclusion that periconceptional multivitamins that contain FA may reduce the risk for CHD, although the effect may be restricted to certain types of CHD.

Folate plays a role in DNA and protein methylation, purine and pyrimidine synthesis and homocysteine conversion to methionine (Bernstein et al., 2007). Polymorphisms in

folate pathway genes such as 5-methyl-tetrahydrofolate-homocysteine methyltransferase (*MTR*), 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*), 5,10-methylenetetrahydrofolate reductase (*MTHFR*) and some located on chromosome 21, such as the cystathionine beta synthase gene (*CBS*) and the reduced folate carrier gene (*SLC19A1*), have been associated with CHDs in several—but not all— studies (van Beynum et al., 2006; Goldmuntz et al., 2008; Brandalize et al., 2009; Mitchell et al., 2010; Locke et al., 2010; Božović et al., 2011; Elsayed et al., 2014).

We use a dataset of 1,722 individuals with DS identified through the population-based National Down Syndrome Project (NDSP) case/control study (using data from Bean et al. (2011)) and from a follow-up Emory Down Syndrome Study (EDSP) based on a convenient sample to test our hypothesis that maternal FA exposure before fetal heart development decreases the occurrence of CHDs in DS probands.

METHODS

Study Population

Two study protocols were used to identify families with infants with Down syndrome (DS) (referred to as probands). The National Down Syndrome Project (NDSP) was a population-based case/control study that enrolled participants from 2001 to 2004 at six sites across the United States (U.S.) that were linked to a birth defects surveillance system. Methods for recruitment and enrollment have been previously reported (Freeman et al, 2007; Freeman et al 2008, Bean et al 2011). The study sample of Bean et al. (2011) was based on this NDSP study sample. The Emory Down Syndrome Study (EDSP) was a

follow-up study based on a convenience sample of families with individuals with DS. Multiple sites across the U.S. used a similar protocol as that used for NDSP to collect data on demographics, maternal health history, clinical data abstracted from medical records and biological samples on the family. This current study includes 859 families with DS enrolled in EDSP and 863 families with DS enrolled in NDSP. Families enrolled in both projects were only included once.

Maternal race and ethnicity was determined by self-reported responses to questionnaires administered to the mother of the proband by trained personnel. Birth records and medical records related to congenital heart defects (CHD) were used to document trisomy 21 and to document heart structure, as described in Freeman et al (2008). Briefly, each site abstracted proband medical records and documented the data on a structured clinical form. This form was reviewed by a clinically trained individual who consulted a pediatric cardiologist as necessary. Each occurrence of a CHD was counted. For this analysis, probands with multiple CHDs were included as cases for the most relevant CHD group. For example, a proband with both complete AVSD and ASD was included in the complete AVSD group. The control group included probands with a structurally normal heart, patent foramen ovale or a patent ductus arteriosus.

Maternal Exposures

Data from the maternal questionnaires were used to determine maternal exposures to prenatal vitamins or supplements, alcohol, cigarettes, gestational diabetes and education. We followed a similar methodology for defining maternal exposures as Bean et al. (2011). The questionnaire asked about prenatal vitamin and supplement use before pregnancy,

during the first three months of pregnancy and after the first three months of pregnancy. Mothers were assigned to three folic acid (FA) groups: exposed, unexposed or uncertain. The exposed group included mothers who began taking prenatal vitamins or supplements containing FA before pregnancy and those who began taking them within first four weeks of pregnancy. The unexposed group included mothers who began taking prenatal vitamins or supplements containing FA after the eighth week of pregnancy and mothers who did not take vitamins or supplements containing folic. The uncertain group included mothers who began taking prenatal vitamins or supplements containing FA during the time of fetal heart development, between the fourth and eighth weeks (Sadler et al., 2005). The uncertain group, along with those who had missing data, was excluded from analysis of FA. Approximately 21% mothers were excluded for beginning prenatal vitamins or supplements containing FA during the time of fetal heart development and 12% were excluded for missing data. The questionnaire asked about alcohol and cigarette exposure during the lifetime, three months before pregnancy and first three months of pregnancy. Mothers who smoked 100 cigarettes or more over their lifetime were considered ever-smokers. Those who smoked at least one cigarette per day between the three months before pregnancy and the first three months of pregnancy were considered exposed to cigarettes. Mothers who consumed one or more alcoholic drinks per week were considered exposed to alcohol. Maternal education level was categorized as either four-year bachelor degree and higher or less than a four-year bachelor degree. Gestational diabetes status was determined by response to a question on presence or absence of gestational diabetes.

Statistical Analysis

Chi-square analysis was conducted to compare frequency distributions across FA groups. Logistic regression was conducted to estimate odds ratios and 95% confidence intervals for the association of CHD with maternal race/ethnicity, proband sex, smoking, alcohol use, gestational diabetes, education and FA supplementation for each CHD group. Infants with Down syndrome without CHD were used as controls. The reported odds ratios were adjusted for maternal race/ethnicity and proband sex based on previous literature. Other covariates were removed from FA models using step-wise removal and a 10% change-in-estimate approach. Interaction terms for FA with any covariate were not significant in FA models in any CHD group. Maternal age at birth, cigarette and alcohol use and gestational diabetes were not significant contributors to FA models in any CHD group. Education and ever-smoke were significantly associated with VSD but were not significant contributors to the FA models in any CHD group. These covariates were removed. One-sided p-values are provided for FA models because the primary hypothesis is that FA supplementation decreases the odds of CHD. Two-sided p-values are provided for covariates. All tests were conducted at the 0.05 significance level. Maternal race/ethnicity and proband sex were significantly associated with many CHD groups and are previously known to be associated with some CHDs among infants with Down syndrome. Therefore, a stratified analysis of FA was conducted for each CHD group using maternal race/ethnicity and proband sex. Statistical analysis was conducted using Statistical Analysis Software (SAS Institute., Cary, NC).

RESULTS

Congenital heart defects by race/ethnicity and sex of proband

The current study population consisted of 1,059 non-Hispanic white mothers (referred to as "white"), 146 non-Hispanic black mothers (referred to as "black") and 438 Hispanic mothers (referred to as "Hispanic") with 873 male probands and 849 female probands. There were too few American Indian/Alaskan Native (n = 11) and Asian (n = 9) mothers to include in the analysis, and 56 mothers who reported "Other" race/ethnicity were also excluded. Families in each CHD group are stratified by maternal demographics and exposures in Table 1.

We first studied the association of race/ethnicity and sex of the proband by CHD subtype. For complete AVSD, logistic regression for maternal race/ethnicity showed a decreased odds ratio (OR) for Hispanics compared to whites (OR = 0.30; p <0.0001), adjusted for proband sex (Table 2). The adjusted OR for blacks compared to whites was not statistically significant. For partial AVSD, the adjusted ORs comparing blacks to whites (OR = 1.91; p = 0.0525) and Hispanics to whites (OR = 0.60; p = 0.0625) were marginally statistically significant. Logistic regression for proband sex and complete AVSD showed an increased OR for females compared to males (OR = 1.75; p <0.0001), adjusted for maternal race/ethnicity. Comparing females to males for partial AVSD resulted in an adjusted OR of 2.45 (p = 0.0001).

Among those with ASD and those with both ASD and VSD, there was an increased OR for Hispanics compared to whites (OR = 1.56; p = .0273 and OR = 1.84; p = 0.0217, respectively), adjusted for proband sex, but no statistically significant association for blacks compared to whites or females compared to males (Table 2). There were no statistically significant associations between maternal race/ethnicity or proband sex with VSD.

Folic acid use as an exposure to heart development

Prior to investigating the association of folic acid (FA) use through prenatal vitamins or supplements during the time of heart development (see Methods) with CHD, we examined this variable by the mother's self-reported racial/ethnic group. First, the proportion of mothers who we categorized as "uncertain" with respect to their FA use was similar across racial/ethnic groups: 21%, 19% and 17% for white, black and Hispanic mothers, respectively (Table 3). This was also the same pattern observed for those with missing data (Table 3). However, the proportion of white, black and Hispanic mothers in the exposed FA group differed significantly from the proportion in the unexposed group: there was a higher proportion of white mothers exposed to FA compared to black or Hispanic mothers (83%, 50% and 39%, respectively; $p < 0.0001$).

The proportion of mothers who we categorized as "uncertain" with respect to their FA use was similar across proband sex groups: 18% for males and 22% for females (Table 3). Among mothers in the exposed and unexposed groups, there was no statistically significant difference in proportion of mothers of male or female probands with FA exposure (66% and 70%, respectively; $p = 0.1915$ Table 3). FA use did not differ among mothers by ever-smoker status, alcohol use or gestational diabetes, after adjusting for maternal race/ethnicity and proband sex (data not shown). FA use was less common among those who used cigarettes during pregnancy (OR = 0.33; $p = 0.0008$) compared to this who did not, and more common among those with a four-year bachelor degree or higher (OR = 3.70; $p < 0.0001$) compared to those with less than a four-year bachelor degree (data not shown).The association of cigarette use and education level with CHD was only significant for the VSD group, however both were non-significant in the VSD FA model, after

adjusting for maternal race/ethnicity and proband sex. Thus, for all the models to examine the association of FA use and CHD, we only adjusted for maternal race/ethnicity and proband sex.

Complete AVSD

Regressing complete AVSD status on maternal FA exposure using DS probands with no CHD as controls, and adjusting for maternal race/ethnicity and proband sex, resulted in a decreased OR for probands with maternal FA exposure compared to those without maternal FA exposure (OR = 0.68; p = 0.0195; Table 2). Interaction terms for FA with maternal race/ethnicity and proband sex were not significant and were removed. Hispanic maternal race/ethnicity and proband sex remained significant in this FA model.

Although the interaction terms were not statistically significant, we further examined the relationship of FA exposure and complete AVSD status by stratifying analyses by maternal race/ethnicity, proband sex and study enrollment (Table 4). The OR comparing probands with maternal FA exposure to those without maternal FA exposure was statistically significant among whites (OR = 0.68; p = 0.0413) but not blacks (OR = 0.67; p = 0.0244) or Hispanics (OR = 0.72; p = 0.2235), adjusted for proband sex. There was no statistically significant association in the stratified analysis for male (OR = 0.67; p = 0.0664) or female probands (OR = 0.69; p = 0.0787), adjusted for maternal race/ethnicity. The association of FA exposure and complete AVSD status was statistically significant when families were stratified by study enrollment. For NDSP, comparing those with maternal FA exposure to those without maternal FA exposure resulted in an OR of 0.63 (p =0.0393),

adjusted for maternal race/ethnicity and proband sex. For EDSP, the same comparison resulted in an adjusted OR of 0.56 ($p = 0.0196$).

Partial AVSD

The same approach was used to regress partial AVSD status on maternal FA exposure using DS probands with no CHD as controls, and adjusting for maternal race/ethnicity and proband sex. Interaction terms for FA were not statistically significant and removed. The association between maternal FA exposure and partial AVSD status was not statistically significant (OR = 0.89; $p = 0.3711$), adjusted for maternal race/ethnicity and proband sex (Table 2). Proband sex remained significant but maternal race/ethnicity did not.

ASD

Controlling for maternal race/ethnicity and proband sex, the same approach resulted in a decreased OR for maternal FA exposure and ASD status (OR = 0.64; $p = 0.0431$). No FA interaction terms were significant. Maternal Hispanic race/ethnicity was associated with ASD status (Table 2) but did not remain significant in this FA model. Maternal black race/ethnicity and proband sex were significant in the model.

VSD

No statistically significant relationship was found between maternal FA exposure and VSD status among DS probands (OR = 0.69; $p = 0.0652$). Ever smoke and maternal

education were associated with VSD status (Table 2) but their interaction terms with FA were not significant. No other FA interaction terms were significant. Maternal race/ethnicity and proband sex were not significant in this FA model.

ASD & VSD

Among DS probands with both ASD and VSD, controlling for maternal race/ethnicity and proband sex, resulted in a decreased OR comparing those with maternal FA exposure to those without FA exposure (OR = 0.50; $p = 0.0179$; Table 2). No FA interaction terms were significant. Maternal race/ethnicity and proband sex were not significant in this FA model.

DISCUSSION

Folic Acid and DS-associated Congenital Heart Defects

Meijer et al. (2006) and Bean et al. (2011) used similar study designs to analyze the effect of maternal for folic acid (FA) supplementation on CHD in live-born infants with DS. Both studies, and our current study, ascertained maternal exposures using a standardized questionnaire and CHD status using medical records. Meijer et al. (2006) defined maternal FA exposure as taking a prenatal vitamin or supplement containing FA, on average at least 4 days a week during the first 12 weeks of pregnancy. Bean et al. (2011) and our current study limited the FA exposure definition to initial of use at least within the first four weeks of pregnancy and excluded from analysis mothers who began taking prenatal vitamins or supplements containing FA during the fifth to eighth week of gestation, a time when heart

development has progressed. Meijer et al. (2006) included probands born before a 1998 U.S. Food and Drug Administration mandate for dietary FA fortification, whereas Bean et al. (2011) included probands born after the mandate. In our study, the vast majority were also born after 1998.

Meijer et al. (2006) included primarily white mothers and found no statistically significant association between maternal FA supplementation and CHD in DS. Bean et al. (2011) included a more racially diverse study population and found maternal FA supplementation was less frequent among DS probands with AVSD and ASD II compared to DS probands with no CHD, and found no statistically significant association between FA and VSD. We included the data from the Bean et al. (2011) study as well as a second cohort of probands with DS, but reclassified the way we assigned probands to CHD subgroups. Whereas Bean et al. included probands with multiple CHD in each relevant CHD group, we categorized probands into only the most relevant (e.g. a proband with both complete AVSD and VSD was only included in the complete AVSD group. We formed an additional group that included probands diagnosed with both ASD and VSD. We showed that maternal FA exposure through prenatal vitamins or supplements during the first four weeks of pregnancy is less common in some CHD groups (AVSD, ASD and ASD/VSD) but not others (partial AVSD and VSD) compared to controls with no CHD.

Folic Acid Association in the Context of Maternal Race/Ethnicity

When we added in the second cohort to the NDSP cohort of Bean et al (2011), we found a higher proportion of white mothers who used FA supplementation during the first four weeks of pregnancy ("exposed") compared to black and Hispanic mothers (83%, 50%

and 39%, respectively). Both studies used similar methods for determining FA exposure status and excluding mothers with uncertain exposure. The pattern of use between cohorts was similar, but proportions found in the combined data were higher than those reported by Bean et al. (2011) (72%, 39% and 27%, respectively), most likely due to increased awareness of the benefits of supplementation with time. The proportion of white, black and Hispanic mothers excluded for uncertain FA exposure was similar in both studies—21%, 19% and 17% in the current study; 21%, 18% and 18% in Bean et al. (2011). These findings suggest the continued need to encourage women to use FA supplementation prior or very early in pregnancy, with targeted efforts toward under-represented minorities.

Despite a lower reported frequency of FA supplementation among Hispanic mothers compared to white mothers, complete AVSD was significantly less common among Hispanic mothers compared to white mothers (Table 2). We found a similar but non-significant pattern among partial AVSD. In contrast, we found statistically significant increased frequency of ASD and both ASD/VSD among Hispanic mothers compared to white mothers. We found no statistically significant association between Hispanic maternal race and VSD. Bean et al. (2011) found a similar pattern for AVSD and VSD, but no statistically significant associations between Hispanic maternal race and ASD. When stratifying by maternal race/ethnicity, we found a significant association of FA with complete AVSD among white mothers and a similar but non-significant association among Hispanic and black mothers (Table 2). Although neither study had data available on specific dietary FA intake, such information may help to understand the added protection against AVSD among the Hispanic mothers compared with white mothers.

Folic Acid Association in the Context of Proband Sex

The risk for both complete and partial AVSD was significantly increased for females with DS compared with males with DS. With respect to FA use, we found no statistically significant interaction terms between FA and proband sex in any CHD group. Consistently, when we stratified the risk of complete AVSD by proband sex, the reduced OR estimate for FA use was the same. Although Bean et al. (2011) did not find a significant interaction term for FA use and proband sex, they did report a statistically significant association between FA and AVSD among males but not females, a statistically significant association between FA and ASD among females but not males, and no significant association between FA and VSD for either males or females. We also found the association of FA with ASD was significant for females (OR = 0.31; $p = 0.0016$) but not males, and the association with both ASD/VSD was significant for males (OR = 0.30; $p = 0.0071$) but not females (data not shown). We found no significant associations of FA with partial AVSD or VSD when stratifying both proband sex. While these findings may suggest etiologic differences, it remains unclear whether they are random effect or represent actual biological differences.

Strengths and Limitations

Both the NDSP and EDSP enrolled DS cases and controls from a live-born population (Freeman et al., 2007). Therefore, in the current study we are unable to analyze the effect of maternal FA supplementation in DS pregnancies that do not result in a live birth. A substantial proportion of pregnancies with DS are lost or electively terminated before birth, with a greater proportion being electively terminated among whites compared

to blacks or Hispanics (de Graaf et al., 2015). These losses early in pregnancy result in a highly-selected DS live birth population. Additionally, infants who were stillborn or died shortly after birth were excluded from both the NDSP and EDSP population (Freeman et al., 2007). While less than 5% of infants were excluded from the NDSP for these reasons, theses infants may have had more severe birth defects than those enrolled.

Strengths of this study include defining maternal FA exposure around the time of the initiation of fetal heart development and the exclusion of those mothers who began taking prenatal vitamins or supplements containing FA during that time. We considered that the latter group may obscure any true associations with FA use. This study also benefitted from a larger sample size than previous studies: we determined maternal FA exposure for 1,180 mothers compared to 799 mothers in Bean et al (2011). A standardized questionnaire was used across all enrollment sites by trained personnel and medical records were used to determine CHD status. Adequate sample sizes allowed us to stratify the AVSD FA model by maternal race/ethnicity and proband sex, but this not possible for the other CHD groups.

Future Work

Our findings contribute to the growing number of studies that associate FA supplementation with decreased CHDs in DS probands. However, these studies have not explained differences in CHD frequency across maternal racial/ethnic groups or included the impact of dietary FA intake. Additionally, the role of FA in contributing to CHD status in DS probands may include genetic interaction. Few, if any, studies have assessed this potential. Those that have done so suffer from small sample sizes (e.g. Locke et al, 2010; Sukla et al., 2015). More studies to identify other risk factors for CHDs, identify and account

for differences in dietary FA intake across racial/ethnic groups and assess environmental and genetic interactions are necessary to better understand how FA impacts CHD status in DS.

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TABLES

Table 1. Proband heart defects stratified by proband sex and maternal exposures

	Control		Complete AVSD		Partial AVSD		ASD		VSD		ASD & VSD	
Exposure	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Proband Sex												
Male	453	(56)	172	(40)	33	(35)	76	(54)	102	(60)	37	(49)
Female	363	(44)	253	(60)	62	(65)	66	(46)	67	(40)	38	(51)
Maternal Race/Ethnicity												
White	480	(59)	322	(76)	61	(64)	68	(48)	95	(56)	33	(44)
Black	55	(7)	46	(11)	14	(15)	11	(8)	12	(7)	8	(11)
Hispanic	235	(29)	47	(11)	18	(19)	52	(37)	56	(33)	30	(40)
Other	45	(6)	10	(2)	2	(2)	11	(8)	5	(3)	3	(4)
Missing	1	(0)	0	(0)	0	(0)	0	(0)	1	(1)	1	(1)
Ever Smoker												
No	600	(74)	308	(72)	80	(84)	109	(77)	123	(73)	59	(79)
Yes	208	(25)	115	(27)	15	(16)	32	(23)	46	(27)	16	(21)
Missing	8	(1)	2	(0)	0	(0)	1	(1)	0	(0)	0	(0)
Current Smoker												
No	759	(93)	396	(93)	89	(94)	134	(94)	160	(95)	73	(97)
Yes	43	(5)	26	(6)	6	(6)	7	(5)	9	(5)	1	(1)
Missing	14	(2)	3	(1)	0	(0)	1	(1)	0	(0)	1	(1)
Alcohol												
No	736	(90)	372	(88)	88	(93)	131	(92)	153	(91)	67	(89)
Yes	70	(9)	47	(11)	7	(7)	9	(6)	15	(9)	7	(9)
Missing	10	(1)	6	(1)	0	(0)	2	(1)	1	(1)	1	(1)
Gestational Diabetes												
No	799	(98)	420	(99)	94	(99)	139	(98)	165	(98)	71	(95)
Yes	17	(2)	5	(1)	1	(1)	3	(2)	4	(2)	4	(5)
Education												
Low	438	(54)	204	(48)	48	(51)	86	(61)	111	(66)	49	(65)
High	378	(46)	221	(52)	47	(49)	56	(39)	58	(34)	26	(35)
Folic Acid												
No	173	(21)	82	(19)	16	(17)	41	(29)	43	(25)	24	(32)
Yes	395	(48)	210	(49)	42	(44)	52	(37)	73	(43)	29	(39)
Uncertain	156	(19)	88	(21)	27	(28)	24	(17)	36	(21)	9	(12)
Missing	92	(11)	45	(11)	10	(11)	25	(18)	17	(10)	13	(17)

Table 2. Logistic regression model results stratified by CHD subtype, adjusted for proband sex and~~/or~~ maternal race/ethnicity

	Complete AVSD		Partial AVSD		ASD		VSD		ASD & VSD	
Exposure	OR_{adj} (95% CI)	*p*	OR_{adj} (95% CI)	*p*	OR_{adj} (95% CI)	*p*	OR_{adj} (95% CI)	*p*	OR_{adj} (95% CI)	*p*
Proband Sex										
Male	-		-		-		-		-	
Female	1.75 (1.37, 2.24)	<0.0001	2.45 (1.55, 3.86)	0.0001	1.13 (0.78, 1.64)	0.5226	0.83 (0.59, 1.17)	0.2953	1.32 (0.81, 2.15)	0.2704
Maternal Race/Ethnicity										
White	-		-		-		-		-	
Black	1.21 (0.79, 1.84)	0.3836	1.91 (0.99, 3.67)	0.0525	1.41 (0.70, 2.82)	0.3386	1.11 (0.57, 2.16)	0.7510	2.12 (0.93, 4.81)	0.0739
Hispanic	0.30 (0.21, 0.42)	<0.0001	0.60 (0.34, 1.03)	0.0625	1.56 (1.05, 2.31)	0.0273	1.2 (0.84, 1.75)	0.3007	1.84 (1.09, 3.09)	0.0217
Folic Acid[a]										
No	-		-		-		-		-	
Yes	0.68 (0.48, 0.98)	0.0195	0.89 (0.46, 1.75)	0.3711	0.64 (0.38, 1.07)	0.0431	0.69 (0.43, 1.11)	0.0652	0.50 (0.26, 0.96)	0.0179

[a]One-sided *p* values are provided for folic acid supplementation

Table 3. Folic Acid Exposure stratified by proband sex and maternal exposures

	Folic Acid Use			
Exposure	No	Yes	Uncertain	Missing
Proband Sex				
Male	24%	47%	18%	12%
Female	20%	47%	22%	12%
Maternal Race/Ethnicity				
White	12%	58%	21%	9%
Black	34%	35%	19%	12%
Hispanic	39%	25%	17%	19%
Current Smoker				
No	18%	52%	21%	10%
Yes	29%	34%	22%	15%
Education				
Low	32%	34%	20%	14%
High	10%	63%	19%	9%

Table 3. Logistic regression model results for the association of folic acid exposure among those with and without complete AVSD, stratified by proband sex, maternal race/ethnicity and study enrollment

Exposure	Folic Acid	Complete AVSD		
		OR_{adj}	p^a	(95% CI)
Proband Sex				
Male	No	-		
	Yes	0.67	0.0664	(0.40, 1.13)
Female	No	-		
	Yes	0.69	0.0787	(0.42, 1.15)
Maternal Race/Ethnicity				
White	No	-		
	Yes	0.68	0.0413	(0.44, 1.05)
Black	No	-		
	Yes	0.67	0.2044	(0.26, 1.74)
Hispanic	No	-		
	Yes	0.72	0.2235	(0.30, 1.68)
Study Enrollment				
NDSP	No	-		
	Yes	0.63	0.0393	(0.38, 1.05)
EDSP	No	-		
	Yes	0.56	0.0196	(0.33, 0.97)

[a]One-sided *p* values are provided for folic acid supplementation