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Preconception folic acid supplementation and risk for chromosome 21 nondisjunction: A report from the National Down Syndrome Project

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Abstract

Both a lack of maternal folic acid supplementation and the presence of genetic variants that reduce enzyme activity in folate pathway genes have been linked to meiotic nondisjunction of chromosome 21; however, the findings in this area of research have been inconsistent. To better understand these inconsistencies, we asked whether maternal use of a folic acid-containing supplement before conception reduces risk for chromosome 21 nondisjunction. Using questionnaire data from the National Down Syndrome Project, a population-based case-control study, we compared the use of folic acid-containing supplements among mothers of infants with full trisomy 21 due to maternal nondisjunction (n=702) and mothers of infants born with no major birth defects (n=983). Using logistic regression, adjusting for maternal age, race/ethnicity, and infant age at maternal interview, we found no evidence of an association between lack of folic acid supplementation and maternal nondisjunction among all case mothers (OR=1.16; 95% CI: 0.90–1.48). In analyses stratified by meiotic stage and maternal age (<35 years or ≥ 35 years), we found an association among older mothers experiencing meiosis II nondisjunction errors (OR=2.00; 95% CI: 1.08–3.71). These data suggest that lack of folic acid supplementation may be associated specifically with MII errors in the aging oocyte. If confirmed, these results could account for inconsistencies among previous studies, as each study sample may vary by maternal age structure and proportion of meiotic errors.

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Keywords

Down syndrome; Trisomy 21; Aneuploidy; Nondisjunction; Chromosome segregation; Folic acid; Meiosis

INTRODUCTION

Folate is an essential B vitamin that provides one-carbon molecules for DNA synthesis, protein synthesis, and methylation of DNA and proteins [Bernstein et al., 2007]. The folate pathway plays a critical role in cellular function and human development, as evidenced by the association between maternal folic acid intake and the risk of neural tube defects (NTD) [Wald and Sheldon, 1991; CDC 1992; Czeizel and Dudas 1992].

James et al. [1999] were the first to suggest a role for the folate pathway in chromosome 21 nondisjunction, the major cause of Down syndrome (DS). They showed that the c.677C>T biochemical changes in folate pathway metabolites in mothers, were associated with a 2.6-fold increased chance of having a child with trisomy 21. Some subsequent studies examining the relationship between maternal folate metabolism and delivering a child with DS found no link with genetic variants in single folate pathway genes, whereas others pointed to an additive effect with variants in multiple genes (for review, see [Coppede, 2009]).

Since maternal folic acid supplementation can modify the effect of genetic variants in the folate pathway, a lack of maternal folic acid supplementation is a potential risk factor for chromosome 21 nondisjunction. Botto et al. [2004] observed a reduced, but not statistically significant, association (OR=0.8; 95% CI: 0.5–1.3) between trisomy 21 and maternal folic acid supplement intake three or more times a week at least one week before conception. Additionally, Czeizel and Puho [2005] found a significantly reduced association (OR=0.5; 95% CI: 0.3–0.9) between trisomy 21 and maternal use of high doses (~6 mg/day) of folic acid in combination with iron during the first month after conception. These authors also found that iron supplementation alone reduced the chance of trisomy 21 (OR=0.4; 95% CI: 0.1–0.9), however the number of women taking folic acid alone was too small to determine its individual effect.

If maternal folic acid supplementation lowers the risk for chromosome 21 nondisjunction, one would expect the prevalence of DS in the United States to drop after the 1998 mandate for folic acid fortification; however, this has not happened to date [Canfield et al., 2005; Collins et al., 2002; Ray et al., 2003; Simmons et al., 2004]. There are several competing factors, such as increasing maternal age at delivery and more widespread use prenatal screening and diagnosis of DS, that could explain the apparent lack of a decrease in the population prevalence of DS.

Environmental factors, such as maternal exposure to folic acid, may influence chromosome segregation at different times during oocyte development. In females, the formation of oocytes is initiated during fetal life and is not completed until decades later (for a detailed description see [Blackburn 2007] and [Nagaoka et al., 2012]). Briefly, meiosis I (MI) begins at around 12 weeks of gestation in the mother's fetal life, at which time chromosomes replicate, and homologs pair and undergo recombination. At approximately 20 weeks in maternal fetal life, MI arrests in prophase, and this arrest is not released until ovulation, some 10 to 50 years later. Upon ovulation, MI is completed, meiosis II (MII) begins immediately, then arrests at metaphase and is completed after fertilization. Thus, studies examining the association between lack of maternal folic acid supplementation and DS in

their offspring examine only the time period between the resumption of MI and the completion of MII.

To date, there have been no studies of maternal folic acid supplementation and chromosome 21 nondisjunction that consider the type of meiotic error. Over 95% of DS results from the failure of chromosomes 21 to properly segregate during meiosis [Mutton et al., 1996]. More than 90% of those meiotic errors are maternal in origin: that is, they occur during formation of oocytes [Freeman et al., 2007; Gomez et al., 2000; Mikkelsen et al., 1995; Yoon et al., 1996]. Among maternal meiotic errors, about 75% occur during MI (e.g., [Antonarakis et al., 1992; Freeman et al., 2007; Mikkelsen et al., 1995; Yoon et al., 1996]), with these percentages naturally being dependent on the maternal age distribution of the population being studied, since advanced maternal age is the most significant risk factor for nondisjunction [Allen et al., 2009]. Interestingly, both MI and MII errors are associated with advanced maternal age [Allen et al., 2009; Antonarakis et al., 1992; Yoon et al., 1996], but the frequency distribution differs, with MII errors being more common than MI errors among the oldest mothers (40–45 years) [Allen et al., 2009].

Altered patterns of recombination along the nondisjoined chromosomes 21 are also associated with nondisjunction. Notably, the patterns of recombination along the nondisjoined chromosome 21 differ significantly between MI and MII errors, implicating different mechanisms and associated risk factors in the nondisjunction [Ghosh et al., 2009; Lamb et al., 2005a; Lamb et al., 2005b; Oliver et al., 2008; Oliver et al., 2011]. Among MI errors, a single telomeric exchange or no exchange is associated with nondisjunction, regardless of the age of the oocyte (i.e., maternal age); among MII errors, it is a pericentromeric exchange that is associated with nondisjunction, and the association increases with the age of the oocyte [Ghosh et al., 2010; Ghosh et al., 2009; Oliver et al., 2008; Oliver et al., 2011].

We used data collected from the National Down Syndrome Project (NDSP), a population-based case-control study of live-born infants with trisomy 21, to test the hypothesis that the lack of maternal folic acid supplementation before conception increases the odds of chromosome 21 nondisjunction. We further explored this association, stratifying by type of meiotic error (MI or MII) and maternal age.

MATERIALS AND METHODS

Study Population

The National Down Syndrome Project (NDSP) is a large, population-based, case-control study that was conducted at six recruitment sites in the United States from 2000 through 2004. The details of the protocols and resulting dataset were described in Freeman et al. [2007]. Briefly, each of the six sites used active birth defects surveillance systems to identify DS live births. These sites included the five central counties of the metropolitan area of Atlanta, Georgia; the states of New Jersey, Iowa, and Arkansas; and selected regions of New York and California.

Cases were defined as live-born infants with free trisomy 21 or mosaic trisomy 21 born to either English- or Spanish-speaking mothers with a primary residence in one of the six study sites. Infants with DS due to translocations and those who were born but died before being enrolled in the study were excluded. Controls were defined as infants without DS or any other major birth defect born to women living in the same geographic areas during the study years [Freeman et al., 2007].

All study sites obtained IRB approval and informed consents from participating families. Maternal questionnaires were administered via telephone by trained study personnel. The date of the interview was used to calculate the age of the infant (in days) at the time of the interview. DNA was extracted from biological samples obtained from case infants and their parents and the parental origin and meiotic stage of the chromosome error were determined using chromosome 21q-specific genetic markers as previously described [Freeman et al., 2007]. Placement of recombination events along 21q was determined by STR and SNP genotyping as previously reported [Oliver et al., 2008; Oliver et al., 2011].

There were 1481 eligible case families and 1716 eligible control families identified through the NDSP. Case participation required obtaining DNA samples from mother and the affected child and administering a questionnaire to the mother. Control participation required completing only the maternal questionnaire. 907 case families (61%) and 983 control families (57%) were enrolled. Participating controls included 977 families reported previously [Freeman et al., 2007], plus an additional six for whom data became available after the 2007 publication.

To examine the effect of maternal use of folic acid supplementation on chromosome segregation at the resumption of meiosis in oocytes, we stratified the cases by meiotic stage of the error. We excluded cases due to paternal errors (n=32), mitotic errors (n=21), mosaics (n=4), maternal errors for which we could not determine stage (n=20), and those for which we could not establish the origin of the error due to limited DNA samples (n=125). Further, we excluded one mother with an MII error who did not complete the maternal questionnaire and two mothers with MI errors whose infants had other chromosome abnormalities, in addition to trisomy 21. Thus, we were left with 702 cases due to maternal meiotic errors: 523 MI and 179 MII and the same 983 controls used in the unstratified analysis.

Preconception Maternal Behavior and Exposures

Self-reported information on maternal age, race/ethnicity, education, and information regarding prenatal vitamin/supplement use, cigarette smoking, and alcohol consumption were gathered via the questionnaire (Table I). Mothers reported their race/ethnicity as white non-Hispanic (referent group), black non-Hispanic, Hispanic, or other. Mothers of “other” race/ethnicity groups were excluded from analyses because of small sample sizes (n=103). Mothers were asked about their prenatal vitamin, vitamin, and supplement use before pregnancy, during the first three months of pregnancy, and after the first three months of pregnancy. Mothers who reported the use of vitamins or supplements other than prenatal vitamins were asked to report the name or brand of the product. All vitamin brands named by mothers contained at least 400 μg of folic acid. All mothers who reported use of a prenatal vitamin, other vitamin, or folic acid were considered to have used a folic acid-containing supplement. For those who began using the product in the first month of pregnancy, the total number of weeks of pregnancy and length of time the product was used determined when use of the product began. Maternal use of a folic acid-containing supplement was defined as follows: “supplemented” for mothers who began using a folic acid-containing supplement before conception, defined as two weeks after the last menstrual period (referent group) and “unsupplemented” for mothers who did not begin using a folic acid-containing supplement before conception; those whose supplement use could not be determined were termed “missing.” Mothers with missing information on folic acid use were excluded (n=10). Maternal education was defined as the highest level attained at the time of completion of the maternal questionnaire; groups were less than a high school education (completing 0–11 years of school) or at least a high school education (referent group). For cigarette smoking and alcohol consumption, mothers were asked questions about use before pregnancy and during the first month of pregnancy. For cigarette smoking, we defined three groups: mothers who smoked more than a half pack of cigarettes per day, mothers who

smoked a half pack of cigarettes per day or less, and mothers who never smoked (referent group). For alcohol consumption, we defined two groups: those who consumed at least one alcoholic drink per week and those who consumed less than one alcoholic drink per week (referent group).

Statistical Analysis

Comparisons between case and control mothers were conducted for categorical and continuous variables. For categorical variables, frequency distributions were compared using the chi-square test. Mean maternal ages at the time of birth of the infant were compared using t-tests. Logistic regression was the primary analysis tool to examine the association of lack of folic acid supplementation and chromosome 21 nondisjunction. All models, including those stratified by maternal age (indicator variable, <35 and ≥35 years of age) were adjusted for maternal age at the time of birth of the infant (continuous variable in years), race/ethnicity (indicator variables), and age of infant at maternal interview (continuous variable in days). Maternal education, cigarette smoking, alcohol consumption, and recruitment site did not result in a meaningful change (>10%) in the odds ratio for the primary exposure variable, folic acid supplementation, and were not considered further. The primary interaction terms involving folic acid use (folic acid by maternal age, folic acid by race/ethnicity, and folic acid by age of infant at maternal interview) were not statistically significant and were not considered further (data not shown).

For all multivariable analyses, we report adjusted odds ratios (ORs) and 95% confidence intervals (CI). Because our primary hypothesis that the lack of folic acid supplementation increases the chance for chromosome nondisjunction is unidirectional, we provide p-values for each OR reflecting a one-sided test at a significance level of 0.05. Results with $p \leq 0.05$ were considered to be of interest. For the more exploratory analyses that enriched for specific recombination profiles, we simply provide two-sided p-values. Statistical analyses were conducted using Statistical Analysis Software (SAS, SAS Institute Inc., Cary, NC).

RESULTS

Study Population

The mean age of case mothers was: 33.6 ± 6.8 years for all maternal errors combined, 33.4 ± 6.6 years for those with only MI maternal errors, and 34.1 ±7.4 years for those with only MII maternal errors. The mean age of control mothers was 28.8 ± 6.2 years (Table I). As shown earlier [Freeman et al., 2007], there were more Hispanic case mothers but fewer black non-Hispanic case mothers versus control mothers (Table I). Consistent with population-based studies [Yang et al., 2007], folic acid-containing supplementation prior to conception also differed by maternal race/ethnicity. Adjusting for maternal age, black non-Hispanic (OR=0.24; 95% CI: 0.16–0.35; p<0.0001) and Hispanic (OR=0.23; 95% CI: 0.18–0.31; p=<0.0001) mothers were less likely than white non-Hispanic mothers to use folic acid. Furthermore, the likelihood of folic acid use increased with maternal age (continuous variable, OR adjusted for race/ethnicity: 1.04; 95% CI: 1.03–1.06; p=<0.0001). Case infants were younger at the time of maternal interview than control infants (285 ± 166 days versus 338 ± 208 days; p<0.0001). Therefore, maternal age, race/ethnicity, and infant age at maternal interview were included as covariates in all models.

Preconception Folic Acid Supplementation by Meiotic Error

There was no association between the lack of folic acid supplementation and nondisjunction for all maternal errors combined (adjusted OR=1.16; 95% CI: 0.90–1.48; p=0.122, Table II). Because of our hypothesis of different risk factors for each type of nondisjunction error, we stratified case mothers by their age group (<35 or ≥35 years of age) and by meiotic stage of

the error (MI or MII) (Table II). Nondisjunction was not associated with lack of maternal folic acid supplementation when case mothers were stratified by either maternal age group or by meiotic error (Table II); however, when cases were stratified by both maternal age and meiotic error, we observed an association between lack of maternal folic acid supplementation and an MII nondisjunction error in older mothers (OR=2.00; 95% CI: 1.08–3.71; p=0.013, Table II). When stratified by maternal race/ethnicity, the estimated ORs for lack of maternal folic acid supplementation and an MII nondisjunction error in older mothers were consistent across groups, but none were statistically significant (Table II).

As an exploratory analysis, we examined the effect of limiting the MII case sample to those with a single pericentromeric recombination event, since the risk for MII errors among those with a pericentromeric recombinant increases with maternal age [Ghosh et al., 2009; Oliver et al., 2008; Oliver et al., 2011]. First, we examined MII cases with a single pericentromeric recombination event, irrespective of the age of the mother (n=90). The association between lack of maternal folic acid supplementation and a MII nondisjunction error in the presence of a single pericentromeric recombinant event was marginally significant (OR=1.77; 95% CI: 1.02–3.06; p=0.020). We further grouped cases by age of the mother. The OR was higher among older mothers (OR=2.06; 95% CI: 1.00–4.23; p=0.024; n=63) compared to younger mothers (OR=1.14; 95% CI: 0.46–2.82; p=0.389; n=27).

DISCUSSION

Based on our previous work suggesting different mechanisms for MI and MII errors [Allen et al., 2009; Lamb et al., 2005a; Lamb et al., 2005b; Oliver et al., 2008; Oliver et al., 2011], we tested the hypothesis that lack of maternal folic acid supplementation around the time of conception increases the risk of chromosome 21 nondisjunction and that this risk may vary depending on the origin of the meiotic error. We restricted our analyses to maternal meiotic nondisjunction errors and stratified by stage of meiotic error and maternal age. We observed an association between MII nondisjunction errors and lack of folic acid supplementation before conception among mothers who were ≥35 years of age (OR=2.00; 95% CI: 1.08–3.71, Table II). There was no association among young mothers with either MI or MII errors. Although these findings need to be confirmed, they raise two important questions: 1) Why is folic acid supplementation associated with only MII errors in older mothers? and 2) Can the restriction of the effect of folic acid to MII errors explain conflicting results from other studies?

If we assume that this age- and error-specific association is true, we suggest that these data provide further evidence that the mechanisms underlying nondisjunction differ and are affected by environmental factors. Several factors differentiate MII errors from MI errors: 1) segregation of sister chromatids at MII occurs during the short period of time between completion of MI at ovulation and fertilization during which time sister chromatid kinetochores must reorient to opposite spindle poles, whereas segregation of homologous chromosomes at MI follows a decades-long metaphase I arrest between maternal fetal life and ovulation [Nagaoka et al., 2012; Watanabe 2012]; 2) MII errors occur more often among very young and among older mothers than do MI errors [Allen et al., 2009]; 3) MII errors are associated with unusual pericentromeric recombination events [Lamb et al., 2005a; Lamb et al., 2005b] that are more prevalent among older than younger mothers [Oliver et al., 2008]; 4) MII errors have been associated with specific environmental factors, although the maternal age pattern sometimes differed. Specifically, Yang et al. [1999] found that smoking and oral contraceptive use around the time of pregnancy were associated with younger mothers with MII errors only. Christianson et al. [2004] found that low SES was associated with MII errors, irrespective of the age of the mother. Ghosh et al. [2010] found that the mean telomere length among older mothers with MII errors was significantly shorter

than among older mothers with MI errors. Although we do not know whether these factors point to a common mechanism, these results suggest that MII errors or the bivalents with specific recombination configurations are differentially sensitive to specific environmental factors, such as folic acid supplementation.

An association of both MI and MII errors with lack of folic acid supplementation could suggest that folic acid helps the fetus with DS to survive to term; however, we observed an effect specific to MII. James et al. [1999] suggested that the environment created by reduced folate in the oocyte leads to hypomethylation. This altered state could compromise a meiotic component needed to segregate chromosome 21 with an unusual pericentromeric recombination event. For example, Rec8, a member of the cohesin complex involved in centromeric cohesion, helps to ensure proper bipolar orientation of homologous chromosomes and stabilize the bivalent [Chiang et al., 2010; Kitajima et al., 2003; Tachibana-Konwalski et al., 2010]. In yeast, Rec8 binds to hypermethylated pericentromeric regions [Kitajima et al., 2003], and studies in mice have shown that cohesin proteins degrade with maternal age [Chiang et al., 2010; Hodges et al., 2005]. If the pericentromeric recombination events more common among MII errors are dependent upon centromeric proteins that degrade with age and are sensitive to environmental exposures, this could explain the association between MII-associated recombination patterns and older maternal age. The chance of improper segregation of chromosomes with at risk recombination patterns could be exacerbated by environmental exposures such as lack of folic acid supplementation.

We also considered whether the association we saw between maternal folic acid supplementation and MII nondisjunction specifically could explain the conflicting results from other studies of folic acid supplementation and the risk of DS. As mentioned, approximately 95% of DS cases are due to chromosome 21 meiotic nondisjunction [Mutton et al., 1996]. The vast majority of cases (~85–95%) are due to maternal errors, with variation in this percentage depending on the maternal age distribution of the study population [Allen et al., 2009; Yoon et al., 1996]. In our study population, 24.0% (230/960) of maternal meiotic cases were MII errors, and 12.8% (123/960) were MII errors in older mothers [Allen et al., 2009]. Based on these data, we roughly estimate that about 11% of live-birth cases of DS are due to MII errors occurring among older mothers. With this low percentage, the power to detect an association with folic acid supplementation is low. If our finding is true, it is not surprising that studies examining changes in the prevalence of DS live births in pre- and post-folic acid fortification detected no differences [Canfield et al., 2005; Collins et al., 2002; Ray et al., 2003; Simmons et al., 2004]. Similarly, case-control studies that defined their outcome as DS, rather than a specific error type, would have low power to detect an association [Botto et al., 2004; Czeizel and Puho 2005].

The strengths of this large population-based study of DS have already been described [Freeman et al., 2007]. The current study capitalized on our ability to stratify the study population by type of chromosome error; however, it also had several important limitations. First, ascertainment by the NDSP was limited to live-born infants with DS. Thus, we are missing up to 80% of trisomy 21 conceptions [Hassold and Jacobs 1984] and have only examined the effect of a lack of folic acid supplementation on pregnancies affected by DS that resulted in a live birth. Second, the maternal history of prenatal vitamin, vitamin, and supplement use was obtained by maternal self-report alone. These data may be inaccurately reported by the mother and may be subject to recall bias; for example, mothers may base their responses on reasons they believe led to an increased risk of having a child with DS. However, as the association was limited to one type of meiotic error, a trait unbeknownst to the mother, this is an unlikely explanation of our results. It is also important to note that, although we were careful to review the folic acid content of specific vitamins and

supplements reported by the mothers, we did not ask for brand names of prenatal vitamins and made the assumption that they, too, contained folic acid. Third, we cannot exclude the possibility that some other component of the vitamins, such as iron as suggested previously [Czeizel and Puho, 2005], contributed to the observed association. Grandmaternal folic acid supplementation was not assessed; therefore, these data cannot be used to test for association of folic acid supplementation and initiation of recombination. Fourth, we were unable to directly measure maternal blood folate levels or test for genetic variants in the folate pathway that influence metabolism. Finally, although our study is the largest to date, our sample sizes were relatively small once we stratified by meiotic error and age group. In particular, the number of older black and Hispanic control mothers was small, hindering the stratified analysis, meaning our data need to be confirmed by an independent sample.

To summarize, we found lack of folic acid supplementation to be associated with MII errors in the aging oocyte, but these observations need to be confirmed in an independent sample. If possible, a more accurate assessment of the folate status around the time of conception, including biomarkers of the folate pathway (e.g., level of homocysteine, plasma and RBC folate levels, etc.), and genotyping of folate genetic variants, will help either confirm or refute these findings.

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TABLE I

Selected Characteristics of Case and Control Mothers

Characteristics	Cases			Controls
	All	Meiotic Error		
		MI	MII	
N	**702**	**523**	**179**	**983**
Maternal age (years) (mean ± SD)	33.6 ± 6.8	33.4 ± 6.6	34.1 ± 7.4	28.8 ± 6.2
Infant age at interview (days) (mean ± SD)	285 ± 166	280 ± 163	302 ± 172	338 ± 208
Age (years)				
<35	345 (49.1%)	271 (51.8%)	74 (41.3%)	808 (82.2%)
≥35	357 (50.9%)	252 (48.2%)	105 (58.7%)	175 (17.8%)
Race/ethnicity				
Non-Hispanic White	364 (51.9%)	269(51.4%)	95 (53.1%)	491 (49.9%)
Non-Hispanic Black	70 (10.0%)	50 (9.6%)	20 (11.2%)	153 (15.6%)
Hispanic	234 (33.3%)	175 (33.5%)	59 (33.0%)	270(27.5%)
Other[a]	34 (4.8%)	29 (5.5%)	5 (2.8%)	69 (7.0%)
Maternal folic acid supplement use				
Supplemented	217 (30.9%)	168 (32.1%)	49 (27.4%)	291 (29.6%)
Unsupplemented	480(68.4%)	351 (67.1%)	129 (72.1%)	687 (69.9%)
Missing	5 (0.7%)	4 (0.8%)	1 (0.6%)	5 (0.5%)
Education				
Less than high school	108 (15.4%)	75 (14.3%)	33 (18.4%)	150 (15.3%)
High school degree or higher	594 (84.6%)	448 (85.7%)	146 (81.6%)	831 (84.5%)
Missing	0	0	0	2 (0.2%)
Cigarette smoking before pregnancy				
> 0.5 packs per day	29 (4.1%)	20 (3.8%)	9 (5.0%)	60 (6.1%)
≤ 0.5 packs per day	56 (8.0%)	41 (7.8%)	15 (8.4%)	92 (9.4%)
No	614 (87.5%)	459 (87.8%)	155 (86.6%)	829 (84.3%)
Missing	3 (0.4%)	3 (0.6%)	0	2 (0.2%)
Cigarette smoking in 1st month of pregnancy				
> 0.5 packs per day	16 (2.3%)	13 (2.5%)	3 (1.7%)	37 (3.8%)
≤ 0.5 packs per day	41 (5.8%)	26 (5.0%)	15 (8.4%)	76 (7.7%)
No	639 (91.0%)	478 (91.4%)	161 (89.9%)	866 (88.1%)
Missing	6 (0.8%)	6 (1.1%)	0	4 (0.4%)
Alcohol consumption before pregnancy				
≥ 1 drink per wk	108 (15.4%)	83 (15.8%)	25 (14.2 %)	207 (21.1%)
< 1 drink per wk	586 (83.6%)	435 (82.9%)	151 (85.8%)	771 (78.4%)

Characteristics	Cases			Controls
	All	Meiotic Error		
		MI	MII	
Missing	7 (1.0%)	7 (1.3%)	0	5 (0.5%)
Alcohol consumption in 1st month of pregnancy				
≥ 1 drink per wk	55 (7.8%)	40 (7.6%)	15 (8.5%)	88 (9.0%)
< 1 drink per wk	637 (90.9%)	479 (91.3%)	158 (89.8%)	889 (90.4%)
Missing	9 (1.3%)	6 (1.1%)	3 (1.7%)	6 (0.6%)

[a] Excluded from further analysis

TABLE II

Association of Lack of Folic Acid Supplementation with the Birth of an Infant with Trisomy 21

All					White				Black				Hispanic			
All maternal meiotic	**Case n (% no suppl.)**	**Control n (% no suppl.)**	**Adjusted OR[a] (95% CI)**	**P-value**	**Case n (% no suppl.)**	**Control n (% no suppl.)**	**Adjusted OR (95% CI)**	**P-value**	**Case n (% no suppl.)**	**Control n (% no suppl.)**	**Adjusted OR (95% CI)**	**P-value**	**Case n (% no suppl.)**	**Control n (% no suppl.)**	**Adjusted OR (95% CI)**	**P-value**
All																
All	**663 (68.3%)**	**910 (70.1%)**	1.16 (0.90, 1.48)	0.122	**361 (54.3%)**	**489 (56.6%)**	1.15 (0.86, 1.54)	0.179	**70 (85.7%)**	**153 (85.6%)**	1.47 (0.63, 3.46)	0.187	**232 (84.9%)**	**268 (85.8%)**	1.08 (0.62, 1.88)	0.394
<35 years[b]	325 (70.8%)	744 (71.4%)	1.10 (0.80, 1.52)	0.269	166 (55.4%)	383 (56.9%)	1.07 (0.73, 1.59)	0.358	35 (91.4%)	124 (87.1%)	2.05 (0.54, 7.74)	0.144	124 (85.5%)	237 (86.5%)	0.93 (0.49, 1.78)	0.415
≥ 35 years	338 (66.0%)	166 (64.5%)	1.02 (0.67, 1.57)	0.460	195 (53.3%)	106 (55.7%)	0.94 (0.58, 1.54)	0.408	35 (80.0%)	29 (79.3%)	0.89 (0.24, 3.32)	0.430	108 (84.3%)	31 (80.6%)	1.65 (0.54, 5.03)	0.190
MI																
All	**490 (66.9%)**	**910 (70.1%)**	1.10 (0.84, 1.43)	0.249	**267 (52.1%)**	**489 (56.6%)**	1.05 (0.76, 1.45)	0.375	**50 (82.0%)**	**153 (85.6%)**	1.12 (0.46, 2.72)	0.405	**173 (85.5%)**	**268 (85.8%)**	1.27 (0.69, 2.34)	0.221
< 35 years	255 (71.4%)	744 (71.4%)	1.20 (0.85, 1.71)	0.147	133 (55.6%)	383 (56.9%)	1.15 (0.76, 1.76)	0.253	30 (90.0%)	124 (87.1%)	1.76 (0.46, 6.73)	0.203	92 (88.0%)	237 (86.5%)	1.21 (0.57, 2.58)	0.311
≥ 35 years	235 (62.1%)	166 (64.5%)	0.81 (0.51, 1.28)	0.184	134 (48.5%)	106 (55.7%)	0.76 (0.45, 1.29)	0.158	20 (70.0%)	29 (79.3%)	0.56 (0.13, 2.38)	0.217	81 (82.7%)	31 (80.6%)	1.42 (0.46, 4.43)	0.273
MII																
All	**173 (72.2%)**	**910 (70.1%)**	1.40 (0.93, 2.09)	0.051	**94 (60.6%)**	**489 (56.6%)**	1.45 (0.90, 2.33)	0.064	**20 (95.0%)**	**153 (85.6%)**	4.70 (0.56, 39.29)	0.076	**59 (83.0%)**	**268 (85.8%)**	0.89 (0.39, 2.02)	0.387
< 35 years	70 (68.6%)	744 (71.4%)	0.81 (0.45, 1.45)	0.242	33 (54.5%)	383 (56.9%)	0.79 (0.37, 1.71)	0.275	5 (100%)	124 (87.1%)	**	**	32 (78.1%)	237 (86.5%)	0.55 (0.22, 1.41)	0.108
≥ 35 years	103 (74.8%)	166 (64.5%)	2.00 (1.08, 3.71)	**0.013**	61 (63.9%)	106 (55.7%)	1.67 (0.83, 3.34)	0.075	15 (93.3%)	29 (79.3%)	3.15 (0.31,32.30)	0.167	27 (88.9%)	31 (80.6%)	2.94 (0.57, 15.28)	0.099

[a]OR, odds ratios; CI, confidence interval; All models, including those stratified by maternal age (indicator variable), are corrected for maternal age (continuous variable), race/ethnicity (indicator variable – full model only), and age of infant at maternal interview (continuous variable)

[b]Maternal age