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Association between maternal periconceptional alcohol use and neural tube defects: Findings from the National Birth Defects Prevention Study, 1997-2011

By

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Degree to be awarded: Master of Public Health

Department of Epidemiology

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Abstract

Association between maternal periconceptional alcohol use and neural tube defects: Findings from the National Birth Defects Prevention Study, 1997-2011

By Adia R. Louden

BACKGROUND: Neural tube defects (NTDs) are major birth defects that occur early in pregnancy. NTDs contribute substantially to fetal and infant mortality and varying degrees of disability worldwide. Risk factors for NTDs include genes and environmental (non-inherited) exposures. Prenatal alcohol exposure has been shown to induce NTDs in animal studies, but results from human studies are mixed. Using data from the National Birth Defects Prevention Study, associations between reports of maternal periconceptional (one month prior and one month following conception) alcohol consumption and NTDs were examined.

METHODS: NTD cases and unaffected live born singleton controls, delivered from 1997 through 2011, were included. Interview reports of alcohol consumption (quantity, frequency, variability, type) were obtained from 2,167 case mothers and 11,728 control mothers. Adjusted odds ratios (aORs) and 95% confidence intervals (CIs) for any and monthly average and maximum average periconceptional consumption were estimated using multivariable logistic regression analysis, controlling for relevant covariables. Similary, aORs were estimated for binge episodes and type of alcohol consumed.

RESULTS: For all NTDs combined, any alcohol consumption, one or more binge episodes, and different types of alcohol consumed were not associated with increased risk for NTDs. Findings were similar for anencephaly and spina bifida subtypes. Modestly increased associations were observed for rare NTD subtypes ($1.1 < aOR < 1.8$). The maximum and periconceptional average monthly drinks were each associated with increased risk for anencephaly cases, when mothers consumed alcohol daily.

CONCLUSIONS: There is no new evidence to suggest an association between any periconceptional alcohol consumption and NTDs; however, there is a possibility that daily drinking during pregnancy might increase the risk of a NTD-affected pregnancy. Underreporting of alcohol consumption in NBDPS interviews may have affected the estimates in this study, due to the negative stigma associated with alcohol consumption during pregnancy. While this study contained rarer NTD subtypes than prior studies, future work should aim to continue to increase sample sizes for these rare subtypes, reduce exposure misclassification, and improve ascertainment of fetal deaths and elective terminations.

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you Granny, here's another brick laid down on my journey until we meet again. I love you dearly.

THESIS STATEMENT

The aim of the current project was to expand on the work previously published by Makelarski et al. (2013). This study included pregnancies from the National Birth Defects Prevention Study (NBDPS) with estimated dates of delivery (EDD)s from 1997-2005 and examined the association between maternal periconceptional alcohol consumption and neural tube defects (NTDs). The current project expanded on this work by including data from NBDPS NTD cases and control with EDDs from 2006-2011 to the previous study years, with a focus on maternal alcohol consumption during the period one month before conception through the first month following conception and NTD subtypes.

KEYWORDS AND ABBREVIATIONS

Keywords: alcohol; anencephaly; birth defects; case-control study; epidemiology; neural tube defects; pregnancy; spina bifida

Abbreviations:

2D	two-dimensional
AFP	alpha-fetoprotein
aORs	adjusted odds ratios
CATI	computer-assisted telephone interview
CI	confidence interval
cORs	crude odds ratios
CNS	central nervous system
DFEs	dietary folate equivalents
EDD	estimated date of delivery
FOCM	folate-mediated one-carbon metabolism
MACDP	Metropolitan Atlanta Congenital Defects Program
µg	microgram
MoM	multiples of the median
MOMS	Management of Myelomeningocele Study
MSAFP	maternal serum alpha-fetoprotein
Mtrr	methionine synthase reductase
NTDs	neural tube defects
NBDPS	National Birth Defects Prevention Study
OR	odds ratio
RR	rate ratio
SB	spina bifida
US	United States

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CHAPTER I

PUBLIC HEALTH OVERVIEW

Neural Tube Defects and their Public Health Significance

Neural tube defects (NTDs) are among the most common major structural birth defects worldwide (Blencowe et al., 2019). NTDs form by the fourth week of gestation, and are associated with substantial morbidity, mortality and life-long disability with varying degrees of paralysis (Botto et al., 1999). The most common NTDs include spina bifida, anencephaly, and encephalocele. Spina bifida occurs when the spinal cord does not develop properly due to incomplete closure of the neural tube. Anencephaly occurs due to failure of complete closure of the neuropore. Encephalocele, occurs when the neural tube does not close along the center of the skull from the nose to the back of the neck. Infants born with spina bifida usually have life-long disabilities accompanied by varying degrees of paralysis, whereas those with anencephaly usually die within a few days following birth (Botto, 1999).

In the United States (US), approximately 3,000 pregnancies are affected by NTDs annually, the majority of which are spina bifida and anencephaly (Williams et al., 2015; Parker et al., 2010). The associated costs of care for these defects are high due to frequent health complications; the most recent estimates of average direct lifetime medical care costs are approximately US$ 800,000 for spina bifida (Grosse et al. 2016) and US$ 6000 for anencephaly (Grosse et al., 2005).

In the early 1990's, randomized trials demonstrated that folic acid has the ability to prevent the development of some NTDs (Czeizel & Dudas, 1992; MRC Vitamin Study Research Group, 1991). These findings led the US Preventive Services Task Force to

recommend that all women planning or capable of pregnancy take a daily multivitamin containing 400 µg to 800 µg of folic acid to prevent NTDs (U.S. Preventive Services Task Force, 2009). Additionally, mandatory folic acid fortification of enriched cereal grain products in the US began in 1998 and continues to be implemented (Williams et al., 2015). Although the prevalence of NTDs decreased in the US following fortification (Williams et al., 2015), other risk factors may explain their persisting prevalence.

Both genetic and non-genetic factors are known to play a role in the development of NTDs. A study by Agopian et al. (2013) reported that the proportion of cases of spina bifida and anencephaly that can be attributed to known risk factors is 28% and 44%, respectively, leaving considerable opportunity for exploration of additional risk factors. To this end, this study sought to comprehensively examine the risk of NTDs associated with maternal consumption of alcohol, a known teratogen, during early pregnancy.

Goal and Significance

The goal of this study was to comprehensively examine the association between periconceptional (one month prior and one month following conception) maternal alcohol consumption and NTDs. The hypothesis was that there is a positive association between maternal periconceptional alcohol consumption and NTDs in their offspring. This study expands on previous work published using the National Birth Defects Prevention Study (NBDPS) data for NTD cases and controls with an estimated date of delivery (EDD) from 1997-2005 (Makelarski et al., 2013), by including data for cases and controls with EDDs from 2006-2011. Pooling data from 1997-2011 provided increased statistical power to test associations and an increased sample size to examine rare subtypes of

NTDs that could not be examined using data from 1997-2005 only (Makelarski et al., 2013).

This study aimed to examine the association between maternal periconceptional (one month prior and one month following conception) maternal alcohol exposure and NTDs in offspring born during 2006-2011, using a multi-state population-based case-control study design.

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

Prevalence of Neural Tube Defects in the United States

Spina bifida and anencephaly, the two most common neural tube defects (NTDs), affect approximately 2,850 pregnancies each year in the United States (US), with a prevalence of 3.7 per 10,000 live births for spina bifida and 2.2 per 10,000 live births for anencephaly (US) (Williams et al., 2015; Parker et al., 2010). The third most common NTD in the US, encephalocele, occurs less frequently, and has a prevalence rate of 0.84 per 10,000 live births (Parker et al., 2010). A previous study that examined the impact of the 1996 US folic acid fortification mandate on NTD prevalence reported significant reductions in the prevalence of spina bifida and anencephaly immediately after fortification (1999-2000); the prevalence rate following fortification has remained relatively stable (Williams et al. 2015). Even though the reductions in the prevalence of spina bifida and anencephaly were observed across all major race/ethnic groups (i.e., non-Hispanic Whites, non-Hispanic Blacks and Hispanics) during early post-fortification years, the prevalence has remained disproportionally higher among Hispanics (Williams et al., 2015), a finding further substantiated by lower folate blood folate concentrations among women of reproductive age in this group compared to non-Hispanic Whites (Tinker et al., 2015).

Screening and Diagnosis of NTDs

In the 1980s, maternal serum screening programs became available to identify pregnancies at risk for NTDs (Wald et al., 1977). The optimal time for NTD screening is between 16-18 weeks of gestation, but screening can also be completed as early as 15

weeks and up to 20 weeks (Bradley et al. 2005). Diagnosis of NTDs is based on biochemical testing and screening of the mother's blood for alpha-fetoprotein (AFP) or through traditional 2-dimensional (2D) ultrasound (Salih, Murshid, and Seidahmed 2014). Fetal serum concentrations of AFP are 150- to 200-times that of amniotic fluid (Habib 1977). This protein represents 90% of total serum globulins in the fetus and leaks into the amniotic fluid for those with an open neural tube (Salih, Murshid, and Seidahmed 2014). In the early 1970s, detection of elevated AFP levels was shown to be associated with open NTDs (Brock, Bolton, and Scrimgeour 1974). Wald et al. (1977) found that maternal serum AFP $\geq$ 2.5 multiples of the median (MoM) occurred in 88% cases of anencephaly, 79% of cases of open spina bifida, and 3% of unaffected singleton pregnancies when tested at 16-18 gestational weeks (Chen 2008). Further, prenatal maternal serum AFP can detect 75% of NTDs during the second trimester of gestation (Wang et al. 2009).

Neural Development

Determining the causes of NTDs is best achieved after understanding the mechanisms underlying neural tube closure (Greene and Copp, 2013). Neurulation begins at approximately 18 days after fertilization, and completion of closure at the posterior neuropore occurs by 26-28 days post-fertilization. Neurulation starts with the flattening of the neuroepithelium that ascends at lateral edges to frame the neural cylinder. The dorsal tips of the neural tube extend and fuse through a process that includes epithelial cell remodeling (Copp, Greene, and Murdoch 2003). The failure of neural plate elevation, neural fold connection, or fusion results in NTDs. Another crucial step of neural tube

development that can cause NTDs when disrupted is specification and separation of cells in the neural tube (Patterson, Waller, and Kroll 2014).

Pathogenetic mechanisms of NTDs

According to Von Recklinghausen (1886), NTDs occur due to an incomplete closure of the cranial and/or caudal neural tube. Morgagni (1769), however, attributed NTDs to increased intraventricular pressure, resulting from excessive cerebrospinal fluid. Morgagni believed this potential mechanism might lead to the reopening of an already closed neural tube, resulting in a NTD. Although most NTDs are reported to result from a primary failure of the closure of the embryonic neural tube, clinical and experimental evidence exists that supports the possibility of a closed neural tube reopening, producing an NTD (O'Rahilly and Muller 2002; Ikenouchi et al. 2002). Experimental and human embryo studies have demonstrated that post-closure defects have a relatively late onset and might occur during an extended time period in development (Padmanabhan 1990; Ikenouchi et al. 2002). Embryology textbooks and review papers describe NTDs as single developmental defects being the direct result of neural tube closure failure rather than being a part of a spectrum maldevelopment affecting the neural tube, meninges, and axial skeletal structures (Van Allen et al. 1993; Van Allen 1996; Copp et al. 2003; Cabrera et al. 2004).

The timing of NTD onset affects not only different regions of the neural tube but also several non-neural organs (Gardner 1980; Seller and Kalousek 1986). Myelomeneningoceles are almost always associated with Chiari II malformations, where parts of the brain bulge through the foramen magnum (McLone and Dias 2003). Seller and Kalousek (1986) compared the frequency and pattern of isolated NTDs with those for

NTDs presenting with other defects. Results showed significant clustering of developmental defects associated with total craniorachischisis and upper thoracic spina bifida. Clustering was less frequent with anencephaly and lumbosacral spina bifida, and did not present with sacral spina bifida. This implies a possible connection between the pathogenetic mechanisms by which NTDs and other defects develop (Seller and Kalousek 1986).

Etiology of NTDs

The etiology of NTDs is thought to be multi-factorial, including genetic and non-genetic factors.

Genetic Factors

Several animal studies suggest that genetic factors are involved in NTD formation. Animal models have shown as many as 100 mutant genes affect neuralation, and almost all of these genes have homologs in humans (Juriloff and Harris, 2000; Klootwijk et al. 2004). Currently, more than 200 genes have been reported to be associated with NTDs in mouse models (Harris and Juriloff, 2010). The substantial number of hereditary defects that contribute to NTDs is due to the number of steps that can possibly be disturbed during the neurulation processes.

Studying the genetics of NTDs in humans poses challenges, due to the lack of available multiplex families, attributed to the high perinatal mortality and morbidity of NTD-affected individuals (Petrini et al., 2002; Pinar, 2004). Nonetheless, several single-gene and chromosomal disorders have been reported to be associated with NTDs (for example, spina bifida occurs regularly in autosomal trisomies). NTDs are also associated with single gene disorders, such as cerebrocostomandibular syndrome, Fraser syndrome,

Meckel-Gruber syndrome, and Waardenburg syndrome. To date, however, no major gene for NTDs has been identified in humans.

Because of the role of folate in NTD prevention, extensive research has been conducted for genes involved in the folate metabolic pathway (Boyles et al. 2006; Morrison et al. 1998; De Marco et al., 2006; Botto and Yang, 2000). More than 25 proteins involved in this pathway have been identified in humans; however, only a few of the corresponding genes to these proteins have been associated with an increased risk of NTDs. To date, detecting moderate effects of multiple folate genes has been difficult due to their potential interaction effects with environmental factors (Boyles et al., 2006).

Family history of NTDs plays an important role in the development of NTDs. In an Alabama study, the overall prevalence of family history of neural tube defects in children with neural tube defect was 16.9%, of which 3.1% were in first-degree relatives (Dupepe et al., 2017). Sex differences have also been observed in the prevalence of NTDs (Liu et al., 2018; Jurilofff and Harris, 2012; Tennant et al., 2011). Liu et al. (2018) found overall NTDs are less prevalent among men than among women (rate ratio (RR) = 0.92, 95% confidence interval (CI) = 0.90, 0.94). Both anencephaly (RR = 0.77, 95% CI = 0.73, 0.81) and encephalocele (RR = 0.75, 95% CI = 0.61, 0.92) were less common among men, while spina bifida (RR = 1.10, 95% CI = 1.05, 1.15) showed a male predominance.

Non-Genetic Factors

Several demographic and non-genetic factors have been associated with NTD prevalence (Agopian et al., 2013). A meta-analysis found an increased risk of having a child with NTDs for mothers over 40 years old as well as mothers less than 19 years old

(Vieira and Castillo, 2005). Hispanic women in the United States have a higher prevalence of NTDs compared to other race/ethnic groups (Williams et al., 2015). Studies have also reported low maternal education to be associated with NTDs in offspring(Little and Elwood, 1992; Canfield, 1996; Wasserman et al., 1998)(Farley et al., 2002). Addtionally, low household SES has been reported to increasethe risk of having NTD-affected offspring (Yang et al., 2008).

Maternal obesity and diabetes have been associated with NTDs (Shaw et al., 1996; Huang et al., 2017). In a recent study, obese women (body mass index (BMI) $\geq$ 30) had twice the odds of having an NTD-affected pregnancy than normal weight women (BMI: 18.0-24.9) (McMahon et al., 2013). Ray et al. (2004) evaluated the risk of congenital anomalies in women participating in an antenatal maternal screening program and reported an increased adjusted odds ratio for NTDs among women with diabetes, although the confidence interval included the null value. Correa et al. (2008) reported that pregestational diabetes mellitus was associated significantly with NTDs, while gestational diabetes mellitus presented weaker associations.

In addition to aforementioned factors, it has been suggested for almost 50 years that maternal folate status is associated with the risk of NTDs (Hibbard, Hibbard, and Jeffcoate, 1965). In a review by Imbard et al. (2013), it was suggested that not only folate status, but complete methylation metabolism could be involved in the etiology of NTDs.

Maternal lifestyle factors, such as smoking and stress, are reported to be associated with epigenetic molecular pathways, leading to abnormal neurological syndrome during childhood. To compare the effects of active smoking and passive smoking during pregnancy on the risk of NTDs, Meng et al. (2018) conducted a meta-

analysis on case-control and cohort studies from 1996 to 2017. The pooled odds ratio (OR) and 95% CI for the risk of NTDs was 1.05 (0.91-1.22) for active smoking and 1.90 (1.56-2.31) for passive smoking (Meng et al., 2018). In a case-control study, mothers who experienced one or more stressful life events during the year before conception had increased risks for NTDs (OR = 2.9; 95% CI = 1.80, 4.70) compared with mothers experiencing no events. Mothers who scored low on emotional support (measured using social integration and perceived emotional support scales) had an elevated risk compared with those who scored high (OR = 4.6; 95% CI = 2.20, 9.70) (Suarez, Cardarelli, and Hendricks 2003).

Certain medications during pregnancy have been reported to be associated with NTDs. Yadzy et al. (2013) reported positive associations between opioid medication use during pregnancy and any NTD and spina bifida, specifically. In another study, therapeutic opioid use was associated with a two-fold increased odds for spina bifida (Broussard et al., 2011). A recent meta-analysis including 28 studies, the pooled risk estimate for the association between maternal exposure to valproic acid and NTDs was reported to be 2.08 (95% CI = 1.55, 2.79) (Tanoshima M et al., 2015).

Association between Maternal Pregnancy-related Alcohol Consumption and NTDs

Alcohol is a known teratogen (Yanaguita et al., 2008). For example, studies of mouse embryos have demonstrated that maternal exposure to alcohol may produce open neural tissue in live and stillborn animals (Randall and Taylor, 1979; Padmanabhan et al., 1994; Becker et al., 1996; Aronne et al., 2008). Alcohol has also been shown to contribute to a wide range of minor neural tube defects following mild doses (Zhou et al., 2003). To date, several mechanisms by which alcohol may influence the development of

NTDs have been proposed. One proposed mechanism is related to excessive cell death of neural crest cells (Bannigan and Burke, 1982). Specifically, exposure to ethanol in pregnant mice beginning at eight-hours post-conception (which corresponds to the third and fourth weeks of human embryonic period during pregnancy), led to excessive cell death of premigratory neural crest cells (Kotch and Sulik, 1992). A subsequent study demonstrated that excessive loss of premigratory neural crest cells inhibits fusion of neural folds (Copp, Greene, and Murdoch, 2003). Another proposed mechanism is that alcohol exposure contributes to folic acid deficiency, thereby playing an indirect role in NTD development (Yanaguita et al., 2008). Alcohol administration in mice resulted in an increase in folic acid excretion in the kidneys followed by a decrease in plasma folate levels (McMartin, 1984; Muldoon and McMartin, 1994). More recent studies have reported mouse embryos exposed to alcohol also showed alterations in DNA methylation, producing NTDs (Liu et al., 2009). Despite all of the aforementioned evidence, the pathogenic mechanism of alcohol remains unclear.

Although animal studies have suggested that alcohol may induce NTDs in offspring, the results of human studies examining the effects of maternal alcohol exposure and NTDs have produced inconsistent results (**Table 1**). A recent meta-analysis examined epidemiological studies of maternal alcohol exposure and NTDs in offspring conducted from 1982 through 2014 (Leng et al., 2016). The meta-analysis included both case-control (n=7) and cohort studies (n=1) and reported a pooled ORs of 1.01 (95% CI = 0.71, 1.45) for all NTDs and 1.03 (95% CI = 0.65, 1.64) for spina bifida. Results examining maternal alcohol consumption the first trimester and binge drinking were similar to those for any alcohol consumption (first trimester alcohol consumption pooled

OR = 1.01 (95% CI = 0.71, 1.43); binge drinking pooled OR = 1.07 (95% CI = 0.81, 1.41). Overall, results were inconclusive for the association between maternal alcohol exposure during pregnancy and NTDs.

There are important limitations in several previous epidemiologic studies of maternal alcohol consumption during pregnancy and NTDs in offspring included in the meta-analysis. Among these limitations were the lack of analyses by NTD subtype (De Marco et al., 2011; Shaw et al., 1996; McDonald et al., 1992; Suarez, 2008), comprehensive analyses of important covariables (McDonald et al., 1992), and investigation of associations by type of alcohol consumed (De Marco et al 2011, Shaw et al., 1996; McDonald et al., 1992; Suarez, 2008). Only one previous study has examined associations by type of alcohol consumed –Makelarski et al., (2013) examined mothers by types of alcohol consumed including: beer only, beer plus other alcohol types (wine and/or distilled spirits), or other alcohol type only. Only a few studies have been able to evaluate relevant covariables due to limited data and/or small sample sizes, including those known to be associated with NTDs such as diabetes and exposure to folate antagonists (Lammer et al., 1987; Becerra et al., 1990). Two studies have examined the association between alcohol consumption and NTD subtypes (Mills and Graubard, 1987; Makelarski et al., 2013). This is important because of potential developmental and etiologic heterogeneity in NTD development (Mitchell, 2005). The inability to stratify by NTD subtype could dilute reported associations due to this underlying heterogeneity (Makelarksi et al., 2013).

Makelarski et al., (2013) sought to address the previously mentioned limitations using data from the National Birth Defects Prevention Study (NBDPS). Associations

between maternal reports of periconceptional (1 month prior through 2 months postconception) alcohol consumption and NTDs were examined. This included examinations of: binge drinking episodes, types of alcohol consumed for all NTDs combined and NTD subtypes. For all NTDs combined, adjusted ORs (aORs) for any alcohol consumption, one or more binge episodes, and different types of alcohol consumed were near unity or modestly reduced ($\geq 0.7 < aOR \leq 1.1$) and were not statistically significant. Findings were similar for individual NTD subtypes (anencephaly, spina bifida, and other rare subtypes such as encephalocele, cranial meningocele, and encephalomyelocele).

Following the study by Makelarski et al., (2013), another multi-center, case-control study examined associations between spina bifida and cigarette, alcohol, and caffeine consumption by women during the first month of pregnancy (Benedum et al., 2013). To measure alcohol consumption, mothers were asked about the average number of drinking days per week (frequency) and the average number of drinks per drinking day (intensity) two months prior to and during pregnancy, including changes in patterns of intake and the date of any such change. Considered separately, neither frequency nor intensity of alcohol use were associated with an increased risk of spina bifida (Benedum et al., 2013). Most aORs for alcohol frequency and intensity in this study were also near unity or modestly reduced ($\geq 0.5 < aOR \leq 1.2$) No other recent, large, population-based studies on the association between alcohol exposure during pregnancy and NTDs were identified.

Building on the previous study by Makelarski et al., (2013), the current study used data from the National Birth Defects Prevention Study (NBDPS) for deliveries from

1997–2011, which included those (1997-2005) examined by Makelarski et al., (2013). Pooling data from 1997–2011 provided a larger sample of cases and allow for the examination of risk of more rare NTD subtypes that could not be examined in the analysis by Makelarski et al., (2013).

CHAPTER III

DESIGN AND METHODS

National Birth Defects Prevention Study

The National Birth Defects Prevention Study (NBDPS) was a multistate case-control study conducted in the United States (US) to examine genetic and environmental factors for major structural birth defects among deliveries from October 1, 1997-December 31, 2011. The NBDPS covered an annual birth population of 482,000 and included case and control deliveries identified by 10 birth defect surveillance programs (Arkansas, California, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, Utah, and metropolitan Atlanta [Georgia]) (Reefhuis et al., 2015). Cases were infants with at least one NBDPS-eligible birth defect diagnosed within the first year of life. Live born control infants were randomly selected from vital records or birth hospital records within the same time period and study region as cases (Reefhuis et al., 2015). Case and control mothers were excluded from the NBDPS if they met one or more of the following criteria: already participated with a previous pregnancy, could not complete the interview, was incarcerated, or did not have legal custody of their infant at the time of the interview (Reefhuis et al., 2015). Eligible mothers were mailed an introductory packet no earlier than six weeks after the infant's estimated date of delivery (EDD). The EDD was used instead of the actual date of delivery to ensure a similar time period between conception and contact of mothers of live births and those of fetal deaths or elective terminations. A standard follow-up protocol that included telephone and mailed reminders was used to answer questions a mother may have had and conduct the NBDPS computer-assisted telephone interview (CATI). NBDPS interviewers used a standardized

script for the CATI to collect data on maternal exposures, including infectious, chemical, physical, nutritional, and behavioral exposures. Interviews were conducted within 24 months following the EDD of an infant. Following the interview, the mother, father, and child (if living) were asked to provide buccal cell specimens by mail using a cytobrush collection kit.

Design and Methods

Study Subjects

Neural tube defect (NTD) cases (British Pediatric Association diagnostic code) included in the NBDPS were anencephaly, including craniorachischisis (740.020, 740.100); spina bifida (741.000-741.990); and encephalocele, including cranial meningocele and encephalomyelocele (742.000-742.090) (Rasmussen et al., 2003). Diagnosis of an NTD was confirmed using standardized criteria and clinical geneticist review of clinical data abstracted from medical records (Rasmussen et al., 2003). If two or more major defects were diagnosed and the defects were developmentally related to one another, then the pattern of defects was classified as isolated. If two or more major defects occurred in different organ systems and the defects did not represent a sequence or complex case, the case was classified as multiple. A complex case was defined as a pattern of major defects that are embryologically related and likely to represent an early problem in morphogenesis. Cases with a chromosomal defect or single gene disorder with known etiology were excluded (Reefhuis et al., 2015).

Exposure

Quantity, frequency, and variability of alcohol consumption was collected during the NBDPS interview. If mothers reported alcohol consumption during the three months

before through the nine months (or fewer for stillbirths or elective terminations) of pregnancy, they were asked about the: month(s) during which they drank (yes/no); average number of drinking days per month (frequency); average number of drinks on one occasion per drinking month (variability); and types of alcohol consumed (beer, wine, and/or distilled spirits). To carry out analyses, a previously developed approach (Romitti et al., 2007) was used to assess maternal periconceptional alcohol consumption during the one-month before (B1) through one-month following conception (P1). Specifically, the average number of drinks per each drinking month was calculated by multiplying the reported average number of drinking days per month by the reported average number of drinks per drinking day for the specified month. During the periconceptional period, the average number of drinks per month was calculated using the average number of drinks per month (B1, P1, both), divided by the number of months a mother drank during the periconceptional period (B1, P1, both). The maximum average number of drinks per month was calculated using the highest reported average number of drinks per month (B1 or P1) divided by the number of months a mother drank during the periconceptional period (B1, P1, both). Four categories of alcohol consumption were used to classify reported periconceptional alcohol consumption using a 30-day month: monthly to weekly (1-4 drinks per month); weekly to every other day (5-15 drinks per month); every other day to daily (16-30 drinks per month); and daily with more than one drink per day (>30 drinks per month).

Binge drinking and binge episodes were estimated using sex-specific (Wechslet et al., 1995) norms. Sex-specific norms for females specify binge drinking as four or more drinks per day on average, on one occasion, or both. To categorize binge drinking, case

and control mothers were classified as: no consumption, consumption without any binge episodes, or one or more binge episodes. Mothers were also classified by types of alcohol consumed, beer, wine, or distilled spirits. The type of alcohol consumed was categorized as: beer only, wine only, distilled spirits, or multiple alcohol types.

Covariables

Covariables evaluated in this analysis included: child sex (male / female), birth weight (<2500 / ≥2500 g), maternal age at delivery (<20 / 20-34 / ≥35 years), race/ethnicity (non-Hispanic white / non-Hispanic black / Hispanic / other), education at delivery (less than high school / high school graduate / college or higher), gravidity (0 / 1-2 / 3+), pre-pregnancy body-mass index (BMI) (underweight: <18.5 / normal: 18.5-24.9 / overweight: 25-29.9 / obese: ≥30 kg/m^2), periconceptional cigarette smoke exposure (active only / passive only / active and passive / none), periconceptional caffeine intake (0-9 / 10-99 / 100-199 / 200-299 / ≥300 mg/day), pre-pregnancy dietary folate intake (<600 / ≥600 μg/day), daily periconceptional folic acid supplement intake (yes / no / unknown), pregnancy intendedness (planned / unplanned), family history of NTDs (yes / no), and history of pre-gestational hypertension (yes / no). To assess dietary folate intake, the Willet Food Frequency questionnaire (Willett *et al.*,., 1985, 1987) was adapted for the NBDPS interview and measured food intake during the one-year before conception, along with reports of breakfast cereals consumed during P1. To estimate dietary folate equivalents (DFEs), reported food frequencies, the standardized serving size, and the United States Department of Agriculture National Standard Reference 16-1 (United States Department of Agriculture 2004) were used.

Vitamin and supplement intake three months before conception through delivery was collected in the NBDPS interview. For each vitamin or supplement reported, mothers were asked to provide start and stop dates (or duration of use if dates were unknown) and frequency of intake. Each reported supplement was assessed to determine whether it contained folic acid. Mothers were classified into two groups: those who took folic-acid-containing supplements during the periconceptional period and those who did not.

Statistical Analysis

Because the exposure period (B1-P1) to be examined in this analysis differs from that (B1–M2) examined in Makerlarski et al., (2013), this analytic dataset includes data from 1997–2011. For the current study, cases classified as isolated or multiple were included in analyses; complex cases were excluded. Mothers with missing responses or who responded unknown for any alcohol use or h more than 150 drinks estimated for average drinking for any month were excluded from analyses. Also excluded were case and control mothers with reported pre-gestational diabetes and/or use of folate antagonist medication (aminopterin sodium, carbamazepine, cholestyramine resin, methotrexate, oxcarbazepine, pyrimethamine, sulfasalazine, triamterene, trimethoprim, phenytoin, primidone, phenobarbital, valproate sodium), as well as case and control mothers with missing or unknown responses for pre-gestational diabetes. Using variables identified as being associated with either NTDs or alcohol exposure in the previous literature, descriptive analyses were conducted comparing NTD cases and controls on relevant child and maternal covariables using the chi-square test of independence. Unadjusted logistic regression models were used to estimate crude odds ratios (cORs) and 95% confidence intervals (CIs) between different categories of maternal alcohol consumption (any,

average drinks per month, maximum drinks per month, binge drinking, type of alcohol) and all NTDs and NTD subtypes. Adjusted odds ratios (aORs) for each category of maternal alcohol consumption were estimated using multivariable logistic regression. Variables were selected using a change-in-estimate approach. For each outcome-alcohol exposure pairing, individual covariables were entered into a model containing the alcohol exposure variable of interest. Covariables which altered the main effect by >20% were retained in the final model. Adjusted odds ratios and 95% CIs were estimated based on the final model between all NTD cases and any maternal alcohol consumption. All analyses were conducted using the Statistical Analysis System (SAS) version 9.4 statistical software (SAS Institute, Cary, NC).

The NBDPS study protocol was approved by the institutional review board at the University of Iowa, Centers for Disease Control and Prevention, and at each participating NBDPS site.

CHAPTER IV

RESULTS

We identified 2,191 NTD cases and 11,829 control infants during the study period 1997–2011. Complete interview data on alcohol consumption were available from mothers of 2,167 NTD cases and 11,728 control infants. We excluded 82 case and 285 control mother interviews due to: complex NTD cases (cases=3); maternal diagnosis of type 1 or type 2 diabetes before or during pregnancy (cases = 50; controls = 81); and reported or unknown maternal periconceptional exposure to folic acid antagonists (cases = 29; controls =204). A total of 2,085 cases were included in our final analysis, including 624 with anencephaly or craniorachischisis, 1,243 with spina bifida, and 218 with encephalocele, including cranial meningocele and encephalomyelocele cases. Our analytic sample included 11,443 controls.

Among all NTD cases, 87.5% were isolated and 12.5% were multiple (**Table 1**). We observed that all NTD-affected infants were significantly different from controls by sex, gestational age, NBDPS site from where they were recruited, maternal race/ethnicity, age at delivery, education at delivery, pre-pregnancy BMI, parity, and cigarette smoking ($p < 0.01$) (Table 2). Comparisons between all NTD-affected infants and controls did not show differences by family history of NTDs, daily folate intake, pregnancy intendedness, periconceptional caffeine intake, and folic acid supplement use ($p > 0.05$) (Table 1).

Descriptive analysis for reported patterns of alcohol consumption showed that a similar proportion of all NTD case and control mothers (31%) reported periconceptional (B1-P1) alcohol consumption (**Table 3**); reported patterns of alcohol consumption were also similar for individual NTD subtypes. The pattern of alcohol use based on the specific

period of periconception (B1 only / B1+P1 / P1 only) was similar between all NTD cases and control mothers (~13%); however, mothers of spina bifida and encephelocele cases reported consumption of alcoholone month before conception (B1 only) more frequently compared to mothers of cases with anencephaly and craniorachischisis. We examined the distribution of type of alcohol consumed, and found that control mothers were more likely to report consumption of beer only, whereas mothers of all NTD cases combined were more likely to report consumption of distilled spirits only. Anencephaly and spina bifida case mothers were more likely to drink beer only and distilled spirits only. Compared to controls, encephalocele case mothers were also more likely to consume distilled spirits (Table 3).

No variable met the criteria for inclusion in an adjusted model. In unadjusted analysis, any periconceptional alcohol consumption was not associated with all NTD cases combined (cOR = 1.01; 95% CI = 0.91, 1.11) (Table 4). The finding did not change when we examined the association by individual or isolated individual subtypes of NTD, with all cORs near unity; no variables met the criteria for inclusion in a multivariable model for all NTDs combined or any NTD subtype, therefore only cORs are presented. Associations between maternal drinking with no binge episodes and maternal drinking with one or more binge episodes (sex-specific standard for females: ≥4 drinks in one sitting) during periconceptional period and all NTD combined were near unity, with similar associations observed for NTD anencephaly and spina bifida subtypes (Table 5). Because no variables met the criteria for inclusion in multivariable models, only cORs are presented for all NTDs combined, anencephaly, and spina bifida. Pregnancy intendedness met the criteria for inclusion in adjusted models for encephalocele cases.

Adjusted analyses for encephalocele, controlling for pregnancy intendedness, also showed no association with one or more binge episodes (aOR = 1.36; 95% CI = 0.82, 2.25) .

Table 6 presents the distribution of drinks per month during the periconceptional period, and the association between frequency of alcohol consumption and NTD outcomes. The proportion of control mothers and all NTD combined case mothers reporting on frequency of average alcohol drinks per month, i.e., 1-4 (14% vs. 14%, respectively), 5-15 (10% vs. 10%, respectively), 16-30 (4% vs. 4%, respectively), >30 (2% vs. 3%, respectively) were similar. An increased frequency of periconceptional average drinks consumed per month measuring >30 drinks per month was associated with all NTD cases combined (cOR = 1.39, 95% CI = 1.05, 1.84) (Table 6). This association was not significant for lower categories of consumption. Further, the positive association between drinking >30 alcoholic drinks per month on average during periconception was positively association with all isolated NTDs (cOR = 1.46; 95% CI = 1.09, 1.95), all and isolated anencephaly and cranioraschisis (cOR = 1.96; 95% CI = 1.30, 2.95 and cOR = 2.04 = 1.33, 3.11, respectively), but no other subtypes of NTD including spina bifida and encephalocele (**Table 6**).

Maximum average monthly drinks exceeding 30 drinks per month were associated with the risk for all NTD cases combined (cOR = 1.48; 95% CI = 1.14, 1.91), and for all isolated NTD cases (cOR = 1.53; 95% CI = 1.16, 2.00). A positive association was also observed for consumption of more than 30 maximum average alcoholic drinks per month and all anencephaly and cranioraschisis (cOR = 2.04; 95% CI = 1.39, 3.00)

and isolated anencephaly and cranioraschisis cases (cOR = 2.15; 95% CI = 1.45, 3.19) (**Table 7**).

Associations between reported types of alcohol consumed during periconception (i.e., beer only, wine only, distilled drinks only, multiple alcohol types) and all NTDs or sub-types of NTDs were mostly near the null; however, ORs were elevated indicating 12%-25% increased odds for selected sub-types of NTDs with maternal consumption of distilled or multiple alcohol types (**Table 8**).

CHAPTER V

DISCUSSION

Our study is one of the largest studies conducted to date, including over 2,000 NTD cases and 11,000 controls identified from the National Birth Defects Prevention Study (NBDPS), a US population-based, case-control study. Findings are based on cases that were confirmed by medical geneticists and have a high diagnostic specificity, and classification by specific phenotypes and isolated case sub-types. Exposure assessment was comprehensive, allowing for analysis by quantity, frequency, variability, and alcohol type. However, we did not find a significant elevated risk of NTDs due to maternal periconceptional exposure to alcohol overall, nor did we observe positive associations between periconceptional average or maximum average monthly drinks and all NTD cases combined. For anencephaly cases, statistically significant, positive associations were observed for mothers who consumed alcohol daily. No significant associations were observed for the spina bifida subtype. Modest, positive associations were observed among isolated cases of the rare NTD subtype, encephalocele (including cranial meningocele and encephalomyelocele cases), across all alcohol analyses. Some elevated odds were observed for specific sub-types of NTDs and type of alcohol consumed. Results examining all isolated cases were generally similar to all NTD cases combined and individual NTD subtypes.

Our study results were similar to those reported in the meta-analysis by Leng et al., (2016), with pooled ORs of 1.01 (95% CI = 0.71, 1.45) for all NTDs and 1.03 (95% CI = 0.65, 1.64) for spina bifida. Additionally, results from our study were similar to

individual studies included in the aforementioned meta-analysis (Mills and Graubard 1987; McDonald et al., 1992; Shaw et al., 1996; Suarez et al., 2008).

While our study observed elevated associations between daily alcohol consumption and NTDs, there was still a lack of association across all other analyses. This lack of association between alcohol consumption and NTDs are inconsistent with previous animal studies (Bannigan and Burke, 1982; Hunter et al., 1994; Zhou et al., 2003; Chen et al., 2005; Yanaguita et al., 2008). As stated previously, the biological mechanisms and pathways by which alcohol affects NTD development are not fully understood. Mouse models suggest that prenatal exposure to alcohol in early development leads to excessive cell death (Bannigan and Burke, 1982; Kotch and Sulik, 1992). This may result in an inadequate number of cells to enable fusion of neural folds (Copp, Greene, and Murdoch, 2003). Mouse models also suggest that prenatal alcohol exposure may play a key role in NTD development by contributing to folic acid deficiency (Yanaguita et al., 2008).

There were no previous studies, not including a study from NBDPS (Makelarski et al., 2013), that examined the association between type of alcohol consumed during pregnancy and NTDs in the offspring. However, exposure to beer was reported to increase red cell folate and plasma 5-MTHFA (Larroque et al., 1992; Stark et al. 2005).

The primary strength of this analysis was the large, diverse sample provided by NBDPS. The comparison of selected maternal characteristics of controls to all live births at each site has shown that NBDPS participants are similar to all live births (Cogswell et al., 2009). Furthermore, all NTD cases were reviewed and verified by clinical geneticists, decreasing the potential for case misclassification. The exposure data was obtained from

detailed maternal interview reports using the NBDPS questionnaire. In addition to its relatively large sample size, another major strength of the NBDPS is the successful collaboration with multi-disciplinary teams across 10 centers in the US (Reefhuis et al., 2015). There is also consistency of study methods across the sites. Specifically, the use of the same case inclusion criteria and interview instrument allows for the creation of a pooled dataset for analysis that is both large and internally consistent. The combination of methodology, sample size, and geographically and racially diverse sample minimizes selection bias. Furthermore, ascertainment bias is reduced by collection of live births (all centers), fetal deaths of 20 weeks or greater gestation (six centers), and elective terminations (five centers) (Yoon et al., 2001).

The overall lack of association between any NTD and maternal periconceptional alcohol consumption in this study may possibly be attributed to methodological limitations, such as the misclassification of timing and dose of alcohol. The exact volume of drinks was not queried on the NBDPS. Instead, general volumes (one can of beer, one glass of wine, and one shot of liquor) were assumed. The varying associations by alcohol type in our study may be due to the differing alcohol concentrations between types of alcohol. Furthermore, alcohol consumption and dose amount were self-reported retrospectively, leading to possible underreporting due to the stigma associated with alcohol consumption during pregnancy. Differential recall of alcohol intake can be an issue between case and control mothers; however, a study by Verkerk et al. (1994) did not find any significant differences in prospective and retrospective reports of alcohol between case and control mothers. The frequency of reported periconceptional alcohol consumption for case and control mothers in the current study, stratified by 6-month

intervals, were found to be similar as the time to interview increased. Another limitation is possibly due to a large proportion of pregnancies affected by NTDs resulting in early fetal deaths (e.g., <20 weeks). This is also possibly another explanation for the lack of association and inconsistent findings between human and animal studies, due to selective early pregnancy loss of fetuses with NTDs, thus causing survival bias (Centers for Disease Control and Prevention, 2004). Cases like these are difficult to include in retrospective case-control studies, because the pregnancy or defect may not be known at the time of loss. Maternal alcohol consumption has also been associated with early pregnancy loss and could also cause this type of bias (Sokol, 1980; Harlap and Shiono, 1980; Rasch, 2003). Nonetheless, the NBDPS includes information on live births, fetal deaths of 20 weeks or greater gestation, and elective terminations, reducing the potential for the aforementioned biases related to case ascertainment.

In conclusion, our study, with a larger sample than that was examined previously using NBDPS, examined associations between maternal periconceptional alcohol consumption and NTDs in their offspring. There was no association between any exposure to periconceptional alcohol and all NTD combined; however, some significant positive associations were observed by increased frequency of consumption and specific types of alcohol, for sub-types of NTDs. These associations should be further studied. Our findings are consistent with the null findings reported in a recent meta-analysis. Our results should be interpreted cautiously, considering reported limitations. Future studies also should aim to improve exposure assessment and increase sample sizes for the rarer sub-types of NTDs, while improving surveillance of NTD-associated fetal deaths and elective terminations for completeness.

Table 1. Study characteristics from studies addressing maternal alcohol consumption and neural tube defects

AUTHOR (YEAR)	TYPE OF STUDY	COUNTRY	STUDY DESIGN	MAIN EXPOSURE	MAIN OUTCOME	RESULTS	ADJUSTED FACTORS
DESROSIERS (2018)	Multi-Center, Population Based NBDPS	USA	Case Control	Carbohydrate Intake	NTDs	Women with restricted carbohydrate intake were more likely to have consumed alcohol in early pregnancy.	Maternal race/ethnicity, education, alcohol use, folic acid supplement use, study center, and caloric intake
SCHMIDT (2018)	Multi-Center, Population Based NBDPS	USA	Case Control	Maternal Caffeine Consumption	NTDs	The association between caffeine consumption and spina bifida was stronger among women who did not consume alcohol (aOR=1.7, CI=1.2,2.4).	Child sex, race/ethnicity, maternal age, smoking, alcohol consumption, and folic acid vitamin intake
ZAHERI (2017)	Prospective Case Control	Iran	Case Control	Risk factors affecting NTDs	NTDs	The history of previous infant with NTDs (OR=10.94; 95% CI=1.75–68.94) and alcohol consumption in the month before and after pregnancy, (OR=14.13; 95% CI=1.15–173.03), are associated (p<0.001) with the incidence of NTDs in infants.	Information not available.
BENEDUM (2013)	Hospital & Birth Defect Registry Based	USA	Case Control	Maternal Cigarette, Alcohol, and Coffee consumption during the first 28 days after last menstrual period (LMP)	SB	SB does not appear to be associated with cigarette smoking, alcohol, or coffee consumption. aOR (95% CI) for highest versus lowest alcohol intake: 1.2 (0.8–2.0)	Study center, maternal education, non-steroidal anti-inflammatory drug use, and use of folic acid antagonist medication
MAKELARSKI (2013)	Multi-Center Population Based NBDPS	USA	Case Control	Maternal reports of periconceptional (1 month prior through 2 months postconception) alcohol consumption	NTDs	Any maternal periconceptional alcohol consumption was not associated with all NTD cases combined. Odds were significantly elevated in mothers of spina bifida cases who reported no folic acid consumption and beer consumption only compared to no alcohol consumption (aOR= 1.8, CI = 1.1– 2.9)	Maternal race/ethnicity, education, pre-pregnancy body mass index, periconceptional smoking, and NBDPS site
DE MARCO (2011)	Hospital Based, Interview/ Questionnaire	Italy	Case Control	Maternal periconceptional factors	SB-affected pregnancies	High caffeine intake (OR = 10.82; 95% CI=3.78–31), low calorie diet (OR = 5.15; 95% CI=1.79–14), occasional consumption of fruit and vegetables (OR = 3.38; 95% CI=1.67–6.82), alcohol consumption (OR = 3.05; 95% CI=1.24–7.50) and lack of folate supplementation at any time of pregnancy (OR = 20.54; 95% CI=5.41–77) mainly determined spina bifida risk.	Information not available.

GREWAL (2008)	Hospital Records Based	USA	Case Control	Periconceptional Maternal Smoking and Alcohol consumption	NTDs, conotruncal heart defects, or orofacial clefts	All NTDs OR (95% CI) for highest versus lowest alcohol intake: 0.6 (0.3–1.4)	Maternal age at the time of conception, body mass index, race/ethnicity, gravidity, employment, and intake of folic acid-containing supplements
SUAREZ (2008)	Multisource Active Surveillance	USA	Case Control	Periconceptional Maternal Cigarette Smoking, Secondhand Smoke, consumption of Alcohol, or use of Street Drugs	NTDs	For women who drank more than one drink daily compared to those who drank none, odds ratios were modestly elevated and overlapped with the null (aOR = 1.5; 95% CI = 0.6, 4.0) for preconception use and 1.3, 95% CI = 0.3, 5.2 for the first trimester). Women who had a binge drinking episode had an aOR of 1.6 (95% CI = 0.8, 3.3)	Maternal age, education, body mass index, dietary intake, and folic acid supplementation
GROENEN (2004)	Multi-Center Population Based	Information not available	Case Control	Role of myo-inositol and zinc, environmental factors and related genes	SB	aOR (95% CI) for highest versus lowest alcohol intake: 0.6 (0.4–1.0)	Information not available.
SHAW (1996)	Hospital & Birth Defect Registry Based	USA	Case Control	Maternal or Paternal Periconceptional use of Recreational Drugs	Neural Tube Defects (NTDs)	Preconceptional use of alcohol as < 1 drink/day (aOR = 0.80, 95% CI=0.62-1.0) or > or = 1 drink/day (aOR = 0.69, 95% CI=0.42-1.2) did not increase the risk for delivering NTD-affected offspring.	Maternal age, race/ethnicity, vitamin use, education, and household income
MCDONALD (1992)	Cohort	Montreal, Quebec, Canada	Retrospective cohort	Cigarette, Alcohol, and Coffee consumption	Congenital defects (chromosomal, neural tube, cleft of lip or palate, cardiovascular, digestive or respiratory, renal or urinary, clubfoot, and musculoskeletal)	The aOR for NTDs was 0.63 in women who took seven or more drinks per week (CI: 0.2-1.7)	Education, ethnicity, maternal age, and cigarette and coffee consumption

CI=Confidence Interval; NBDPS=National Birth Defects Prevention Study; OR=Odds Ratio; SB=Spina Bifida; USA=United States of America

Table 2. Characteristics of infants and birth mothers by control and case subtype, National Birth Defects Prevention Study, 1997-2011

Characteristic	Controls (n= 11443)		All NTD Cases Combined (n=2085)		Anencephaly and Cranioarchischisis (n=624)		Spina Bifida (n=1243)		Encephalocele (n=218)	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Child										
Phenotype										
Isolated	NA	NA	1824	87.48	559	89.58	1102	88.66	163	74.77
Multiple	NA	NA	261	12.52	65	10.42	141	11.34	55	25.23
Sex[a,c]										
Male	5839	51.03	965	46.28	248	39.74	617	49.64	100	45.87
Female	5592	48.87	1006	48.25	294	47.12	600	48.27	112	51.38
First Degree Family History of NTDs										
No	11419	99.79	2062	98.90	621	99.52	1228	98.79	213	97.71
Yes	24	0.21	23	1.10	3	0.48	15	1.21	5	2.29
Gestational Age (weeks)[a,b,c]										
Preterm: <37 weeks	1046	9.14	894	42.88	457	73.24	341	27.43	96	44.04
Term: ≥37 weeks	10397	90.86	1191	57.12	167	26.76	902	72.57	122	55.96
NBDPS Site[a,b,c]										
Arkansas	1426	12.46	248	11.89	75	12.02	145	11.67	28	12.84
California	1224	10.70	390	18.71	133	21.31	224	18.02	33	15.14
Iowa	1266	11.06	237	11.37	69	11.06	146	11.75	22	10.09
Massachusetts	1361	11.89	109	5.23	23	3.69	74	5.95	12	5.50
New Jersey	567	4.95	69	3.31	10	1.60	51	4.10	8	3.67
New York	957	8.36	103	4.94	17	2.72	72	5.79	14	6.42
Texas	1362	11.90	249	11.94	82	13.14	143	11.50	24	11.01
CDC/Atlanta	1202	10.50	264	12.66	82	13.14	154	12.39	28	12.84
North Carolina	989	8.64	207	9.93	74	11.86	105	8.45	28	12.84
Utah	1089	9.52	209	10.02	59	9.46	129	10.38	21	9.63
Maternal										
Race/Ethnicity[a,b,c]										
Non-Hispanic White	6622	57.87	1067	51.18	308	49.36	669	53.82	90	41.28
Non-Hispanic Black	1261	11.02	187	8.97	52	8.33	97	7.80	38	17.43
Hispanic	2817	24.62	695	33.33	220	35.26	406	32.66	69	31.65
Other	737	6.44	136	6.52	44	7.05				
Age at Delivery (years)[a,c]										
<20	1142	9.98	223	10.70	70	11.22	123	9.90	30	13.76
20-34	8701	76.04	1591	76.31	474	75.96	959	77.15	158	72.48
≥35	1600	13.98	271	13.00	80	12.82	161	12.95	30	13.76
Education at Delivery (years)[a,c]										
Less than high school	1843	16.11	408	19.57	136	21.79	220	17.70	52	23.85
High school graduate	2630	22.98	547	26.24	159	25.48	327	26.31	61	27.98
College or higher	6639	58.02	1071	51.37	312	50.00	661	53.18	98	44.95
Pre-pregnancy BMI (kg/m^2)[a,c]										
Underweight (<18.5)	580	5.07	79	3.79	30	4.81	42	3.38	7	3.21
Normal Weight (18.5-24.9)	5847	51.10	930	44.60	291	46.63	527	42.40	112	51.38
Overweight (25- <30.0)	2489	21.75	465	22.30	142	22.76	290	23.33	33	15.14
Obese (≥30.0)	1992	17.41	470	22.54	126	20.19	296	23.81	48	22.02
Parity[a,c]										
0	3520	30.76	653	31.32	200	32.05	394	31.70	59	27.06
1-2	5440	47.54	978	46.91	299	47.92	569	45.78	110	50.46
≥3	2479	21.66	453	21.73	125	20.03	279	22.45	49	22.48
Daily Folate Intake (µg/day)										
<600	8031	70.18	1456	69.83	432	69.23	876	70.47	148	67.89
≥600	3407	29.77	629	30.17	192	30.77	367	29.53	70	32.11
Pregnancy Intendedness										

Yes	4984	43.56	886	42.49	250	40.06	546	43.93	90	41.28
No	3207	28.03	576	27.63	182	29.17	336	27.03	58	26.61
Periconceptional Caffeine Intake (mg/day)										
0-9	1482	12.95	269	12.90	81	12.98	158	12.71	30	13.76
10-99	4197	36.68	740	35.49	220	35.26	443	35.64	77	35.32
100-199	2841	24.83	536	25.71	162	25.96	327	26.31	47	21.56
200-299	1445	12.63	293	14.05	81	12.98	177	14.24	35	16.06
≥300	1427	12.47	235	11.27	77	12.34	131	10.54	27	12.39
Cigarette Smoking[a,b,c]										
No Active or Passive Smoking	7821	68.35	1366	65.52	421	67.47	812	65.33	133	61.01
Active Smoking Only	852	7.45	120	5.76	21	3.37	83	6.68	16	7.34
Passive Smoking Only	1309	11.44	319	15.30	100	16.03	179	14.40	40	18.35
Active and Passive Smoking	1125	9.83	171	8.20	32	5.13	118	9.49	21	9.63
Use of Folic Acid-Containing Supplements										
Yes	1366	11.94	284	13.62	81	12.98	169	13.60	34	15.60
No	9789	85.55	1732	83.07	521	83.49	1037	83.43	174	79.82

BMI, body mass index; CDC, Centers for Disease Control and Prevention; NA, not applicable; NTD, neural tube defect

[a]Numbers may vary due to incomplete or missing data.

[b]Because of rounding, percentages may not total 100.

[c]$p < 0.01$ for all NTD cases combined compared to controls.

[d]$p < 0.05$ for all NTD cases combined compared to controls.

Table 3. Reported Patterns of Maternal Periconceptional Alcohol Consumption and Type of Alcohol Consumed, National Birth Defects Prevention Study, 1997–2011

Periconceptional Alcohol Consumption[a]	Control infants (N=11443)		ALL NTD cases combined (N=2085)		Anencephaly and Craniorachischisis (N=64)		Spina Bifida (N=1243)		Encephalocele[e] (N=218)	
	N	**%[b]**	**N**	**%[b]**	**N**	**%[b]**	**N**	**%[b]**	**N**	**%[b]**
Any consumption[c]										
Yes	3495	30.54	641	30.74	199	31.89	370	29.77	72	33.03
No	7762	67.83	1417	67.96	418	66.99	856	68.87	143	65.60
Period of Periconceptional Consumption[c]										
B1 only	1574	13.76	268	12.85	68	10.90	168	13.52	32	14.68
B1+P1	1420	12.41	285	13.67	96	15.38	159	12.79	30	13.76
P1 only	501	4.38	88	4.22	35	5.61	43	3.46	10	4.59
Type of alcohol[d]										
Beer Only	916	8.00	154	7.39	50	8.01	89	7.16	15	6.88
Wine Only	658	5.75	118	5.66	31	4.97	75	6.03	12	5.50
Distill Only	874	7.64	171	8.20	50	8.01	98	7.88	23	10.55
Beer + wine	268	2.34	65	3.12	17	2.72	41	3.30	7	3.21
Beer + distill	383	3.35	67	3.21	20	3.21	40	3.22	7	3.21
Wine + distill	227	1.98	39	1.87	19	3.04	15	1.21	5	2.29
Beer+wine+distill	156	1.36	25	1.20	12	1.92	11	0.88	2	0.92

NTD, neural tube defect.

[a]Excluded mothers with incomplete or missing alcohol consumption data for any month and mothers who reported > 150 drinks for any month

[b]Because of rounding, percentages may not total 100.

[c]Missing incomplete or questionable data on alcohol consumption were distributed as follows: controls (N = 186), all NTD cases combined (N=27), anencephaly and craniorachischisis (N=5), spina bifida (N=13), and encephalocele (N=1).

[d]Missing, incomplete or questionable data on alcohol type were distributed as follows: controls (N=353), all NTD cases combined (N=64), anencephaly (N=20), spina bifida (N=40), and encephalocele (N=4).

[e]Encephalocele cases include: cranial meningocele and encephalomyelocele.

Table 4. Association of Maternal Reports of Any Periconceptional Alcohol Consumption with Risk of Neural Tube Defect Subtypes and Phenotype, National Birth Defects Prevention Study, 1997–2011

	None	Any Consumption		
	N	N	Odds Ratio	95% Confidence Interval
Controls	7762	3495	Ref	
All NTD cases combined	1417	641	1.01	0.91, 1.11
Isolated	1227	257	1.04	0.93, 1.15
Anencephaly and Craniorachichisis	418	199	1.06	0.89, 1.26
Isolated	372	370	1.08	0.90, 1.30
Spina Bifida	856	370	0.96	0.85, 1.09
Isolated	756	160	0.97	0.85, 1.11
Encephalocele[c]	143	72	1.12	0.84, 1.49
Isolated	99	24	1.37	0.99, 1.89

NTD, neural tube defect

[a]Excluded mothers with incomplete or missing alcohol consumption data for any month and mothers who reported > 150 drinks for any month

[b]Missing incomplete or questionable data on alcohol consumption were distributed as follows: controls (N =186), all NTD cases combined (N=27), anencephaly and craniorachischisis (N=7), spina bifida (N=17), and encephalocele (N=3).

[c]Encephalocele cases include: cranial meningocele and encephalomyelocele.

Table 5. Association of Maternal Reports of Alcohol Binge Episodes with Risk of Neural Tube Defect Subtypes and Phenotype, National Birth Defects Prevention Study, 1997–2011

	0 drinks/month	No binge episodes			One or more binge episodes		
	N	N	Odds ratio	95% Confidence interval	N	Odds ratio	95% Confidence interval
Controls	7762	2080	Ref		1359	Ref	
All NTD cases combined	1417	379	1.00	0.88, 1.13	247	1.00	0.86, 1.15
Isolated	1227	338	1.03	0. 90, 1.17	223	1.04	0.89, 1.21
Anencephaly and Craniorachichisis	418	112	1.00	0.81, 1.24	82	1.12	0.88, 1.43
Isolated	372	102	1.02	0.82, 1.28	76	1.17	0.91, 1.50
Spina Bifida	856	226	0.99	0.84, 1.15	134	0.89	0.74, 1.08
Isolated	756	204	1.01	0.86, 1.18	118	0.89	0.73, 1.09
Encephalocele[c,d]	143	41	0.93	0.62, 1.40	31	0.98	0.61, 1.58
Isolated	99	32	1.03	0.64, 1.66	29	1.36	0.82, 2.25

NC, not calculated; NTD, neural tube defect; Ref, reference.

[a]Missing, incomplete, or questionable data for consumption were distributed as follows controls (N=242), all NTD cases combined (N=42), anencephaly and craniorachichisis (N=12), spina bifida (N=27), encephalocele (N=3).

[b]Binge episode defined by sex-specific standards, ≥ 4 drinks in one sitting.

[c]Encephalocele cases include: cranial meningocele and encephalomyelocele.

[d]Encephalocele cases adjusted for pregnancy intendedness.

Table 6. Association of Maternal Reports of Periconceptional Average Alcoholic Drinks Consumed Per Month with Risk of Neural Tube Defect Subtypes and Phenotype, National Birth Defects Prevention Study, 1997–2011

	Drinks per month												
	0	**1-4**			**5-15**			**16-30**			**>30**		
	N	**N**	**Odds Ratio**	**95% Confidence Interval**	**N**	**Odds Ratio**	**95% Confidence Interval**	**N**	**Odds Ratio**	**95% Confidence Interval**	**N**	**Odds Ratio**	**95% Confidence Interval**
Controls	7762	1601	Ref		1126	Ref		451	Ref		256	Ref	
All NTD cases combined	1417	285	0.98	0.85, 1.12	199	0.97	0.82, 1.14	77	0.94	0.73, 1.20	65	1.39	1.05, 1.84
Isolated	1227	259	1.02	0.89, 1.18	178	1.00	0.84, 1.18	65	0.91	0.70, 1.19	59	1.46	1.09, 1.95
Anencephaly and Craniorachichisis	418	80	0.93	0.73, 1.19	61	1.01	0.76, 1.33	26	1.07	0.71, 1.61	27	1.96	1.30, 2.95
Isolated	372	73	0.95	0.74, 1.23	55	1.02	0.76, 1.36	25	1.16	0.76, 1.75	25	2.04	1.33, 3.11
Spina Bifida	856	176	1.00	0.84, 1.18	114	0.92	0.75, 1.13	37	0.74	0.53, 1.05	33	1.17	0.81, 1.69
Isolated	756	162	1.04	0.87, 1.24	101	0.92	0.74, 1.14	29	0.66	0.45, 0.97	30	1.20	0.82, 1.77
Encephalocele[b,c]	143	29	0.84	0.52, 1.35	24	0.89	0.52, 1.52	14	1.66	0.88, 3.10	5	0.75	0.24, 2.38
Isolated	99	24	0.98	0.57, 1.68	22	1.21	0.68, 2.13	11	1.75	0.84, 3.64	4	1.10	0.34, 3.50

NC, not calculated; NTD, neural tube defect; Ref, reference.

[a]Missing, incomplete, or questionable data for consumption were distributed as follows controls (N=242), all NTD cases combined (N=42), anencephaly and craniorachichisis (N=12), spina bifida (N=27), and encephalocele (N=3).

[b]Encephalocele cases include: cranial meningocele and encephalomyelocele.

[c]Encephalocele cases adjusted for pregnancy intendedness.

Table 7. Association of Maternal Reports of Maximum Average Alcoholic Drinks Consumed Per Month with Risk of Neural Tube Defect Subtypes and Phenotype, National Birth Defects Prevention Study, 1997–2011

	Drinks per month											
	0	1-4			5-15			16-30			>30		
	N	N	Odds ratio	95% Confidence interval	N	Odds ratio	95% Confidence interval	N	Odds ratio	95% Confidence interval	N	Odds ratio	95% Confidence interval
Controls	7762	1579	Ref		1104	Ref		469	Ref		282	Ref	
All NTD cases combined	1417	281	0.98	0.85, 1.12	193	0.96	0.81, 1.13	76	0.89	0.69, 1.14	76	1.48	1.14, 1.91
Isolated	1227	257	1.03	0.89, 1.19	170	0.97	0.82, 1.16	66	0.89	0.68, 1.16	68	1.53	1.16, 2.00
Anencephaly and Craniorachichisis	418	79	0.93	0.73, 1.19	59	0.99	0.75, 1.31	25	0.99	0.65, 1.50	31	2.04	1.39, 3.00
Isolated	372	73	0.97	0.75, 1.25	52	0.98	0.73, 1.32	24	1.07	0.70, 1.63	29	2.15	1.45, 3.19
Spina Bifida	856	173	0.99	0.84, 1.18	111	0.91	0.74, 1.12	38	0.74	0.52, 1.03	38	1.22	0.87, 1.73
Isolated	756	160	1.04	0.87, 1.24	97	0.90	0.72, 1.12	32	0.70	0.49, 1.01	33	1.20	0.83, 1.74
Encephalocele[b,c]	143	29	0.85	0.52, 1.36	23	0.85	0.49, 1.48	13	1.45	0.76, 2.80	7	1.12	0.45, 2.77
Isolated	99	24	0.99	0.58, 1.70	21	1.15	0.64, 2.07	10	1.48	0.68, 3.22	6	1.63	0.66, 4.07

NC, not calculated; NTD, neural tube defect; Ref, reference.

[a]Missing, incomplete, or questionable data for consumption were distributed as follows controls (N=247), all NTD cases combined (N=42), anencephaly and craniorachichisis (N=12), spina bifida (N=27), and encephalocele (N=3).

[b]Encephalocele cases include: cranial meningocele and encephalomyelocele.

[c]Encephalocele cases adjusted for pregnancy intendedness.

Table 8. Association of Maternal Reports of Alcohol Type with Risk of Neural Tube Defect Subtypes and Phenotype, National Birth Defects Prevention Study, 1997–2011

	0 drinks per month N	Beer only			Wine Only			Distill Only			Other Mixed Drinks		
		N	OR	95%CI	N	OR	95% CI	N	OR	95%CI	N	OR	95% CI
Controls	7762	916	Ref		658	Ref		874	Ref		1034	Ref	
All NTD cases combined	1417	154	0.92	0.77, 1.10	118	0.98	0.80, 1.20	171	1.07	0.90, 1.27	196	1.04	0.88, 1.22
Isolated	1227	134	0.93	0.76, 1.12	107	1.03	0.83, 1.27	155	1.12	0.94, 1.34	175	1.07	0.90, 1.27
Anencephaly and Craniorachichisis	418	50	1.01	0.75, 1.37	31	0.88	0.60, 1.27	50	1.06	0.79, 1.44	68	1.22	0.94, 1.59
Isolated	372	46	1.05	0.77, 1.43	27	0.86	0.58, 1.28	47	1.12	0.82, 1.53	61	1.23	0.93, 1.63
Spina Bifida	856	89	0.88	0.70, 1.11	75	1.03	0.81, 1.33	98	1.02	0.82, 1.27	107	0.94	0.76, 1.16
Isolated	756	76	0.85	0.67, 1.09	71	1.11	0.86, 1.43	88	1.03	0.82, 1.30	95	0.94	0.75, 1.18
Encephalocele[c,d]	143	15	0.92	0.51, 1.63	12	0.72	0.33, 1.55	23	0.99	0.56, 1.77	21	0.99	0.57, 1.70
Isolated	99	12	1.13	0.60, 2.13	9	0.75	0.30, 1.86	20	1.24	0.66, 2.34	19	1.25	0.69, 2.25

NC, not calculated; NTD, neural tube defect.
[a]Numbers may vary due to incomplete or missing data.
[b]Missing, incomplete, or questionable data for consumption were distributed as follows controls (N=), all NTD cases combined (N=), anencephaly and craniorachichisis (N=), spina bifida (N=), and encephalocele (N=).
[c]Encephalocele cases include: cranial meningocele and encephalomyelocele.
[d]Encephalocele cases adjusted for pregnancy intendedness.

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