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Maternal Pregestational Diabetes Mellitus and Neural Tube Defects in the Offspring:

Findings from Nationwide Registries in Finland from 2000-2014

By

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MPH, Emory University, 2019

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Maternal Pregestational Diabetes Mellitus and Neural Tube Defects in the Offspring:

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Bachelor of Science
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2017

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2019

Abstract

Maternal Pregestational Diabetes Mellitus and Neural Tube Defects in the Offspring:

Findings from Nationwide Registries in Finland from 2000-2014

By Sanjida Mowla

BACKGROUND: Neural tube defects (NTDs) are the second most common birth defects globally and contribute to child morbidity and mortality. Pregestational diabetes mellitus (PGDM) has been reported as one of the risk factors for NTDs. The purpose of this study was to estimate the prevalence of PGDM and its association with NTDs, including spina bifida, anencephaly, and encephalocele, among live births and stillbirths in Finland.

METHODS: We conducted a population-based case-control study in Finland including live births and stillbirths occurring from 2000-2014. We linked three national health registers: the Finnish Hospital Discharge Register (FHDR), the Medical Birth Register (MBR) and the Register of Congenital Malformations (RCM). We also used census data from Statistics Finland. We estimated prevalence of PGDM, and its association with any NTDs (n=240) and isolated NTDs (n=144). The association between PGDM and NTDs was assessed using multivariable logistic regression. We estimated adjusted odds ratios (aORs) and 95% confidence intervals using multivariable logistic regression.

RESULTS: Of the 876,912 births considered for this study, 240 (0.03%) births resulted in an NTD while the remaining 876,672 (99%) live births and stillbirths without NTDs were considered to be controls in our analysis. Of the births examined, 4,112 births (0.47%) occurred among mothers with PGDM; 0.47% of control births were affected by PGDM while 1.67% of case births were affected by PGDM. Among cases, 144 (60%) births had an isolated NTD. After adjusting for potential confounders, PGDM was significantly associated with any NTD (aOR=3.25; 95% CI: 1.03, 10.27). This association was marginally significant for isolated NTDs (aOR=3.34; 95% CI: 0.82, 13.69).

CONCLUSIONS: Our analyses indicate that PGDM is significantly associated with NTDs among live births in Finland. Data on all birth outcomes, including terminations, is necessary for future studies examining this association in Finland. Meanwhile, PGDM should be monitored and timely treatments engaged. Proper glucose control and intake of folic acid before and during pregnancy is an effective preventative measure that should be encouraged among women of reproductive age in Finland.

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I would like to thank the Bill and Melinda Gates Foundation for supporting me through my undergraduate and graduate education. I was able to explore public health through the Gates Millennium Scholarship Program, and this foundation has allowed me to achieve more than I thought possible. I would like to thank Dr. Vijaya Kancherla for allowing me to explore my interests in birth defects through this thesis topic, and for being such a responsive advisor. I would also like to thank Dr. Mika Gissler and Dr. Sari Räisänen for taking the time to assist me with this project all the way from Finland, and to the staff of the Finnish Medical Birth Register for allowing me to use data for this study. I want to thank the Epidemiology Department for providing students with amazing resources, and ultimately making me genuinely more interested in Epidemiology. Finally, I would like to thank my parents and sisters for their encouragement.

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CHAPTER 1

OVERVIEW AND PUBLIC HEALTH SIGNIFICANCE

Multiple studies have shown that women with pregestational diabetes mellitus (PGDM) are at an increased risk of having their pregnancies affected by birth defects; especially neural tube defects (NTDs). This is particularly of concern in Finland, where there is an increased prevalence of type 1 diabetes. Finland, among a few other European countries, lacks mandatory folic acid fortification, a known intervention for primary prevention of NTDs. NTDs are highly fatal and lead to lifelong disabilities and morbidity among those that survive. In many countries in Europe, including Finland, prenatal diagnosis of NTDs results in a high proportion of elective terminations. The cost of care for those living with NTDs is high, and it has a high economic and psychological impact on affected families. The association between maternal PGDM and the odds of having an offspring with a NTD has not been studied in Finland. This knowledge will help to develop prevention and education programs to address PGDM and NTDs in the country.

The European Surveillance of Congenital Anomalies (EUROCAT) is a network that is invested in surveying population-based prevalence rates of NTDs in several European birth defects registries, including Finland. According to a systematic review using data obtained from EUROCAT, the prevalence of all NTDs in Finland from 2003-2011 was 9.0 (95% CI: 8.3, 9.9) per 10,000 births and 4.0 (95% CI: 3.5, 4.6) for spina bifida. Using data collected from 1990-2014, the overall prevalence of NTDs in Europe ranged from 1.3 to 35.9 per 10,000 births [1]. The EUROCAT online data generator was used to

estimate the prevalence rates of NTDs in Finland from 2000-2014. The prevalence of NTDs excluding genetic conditions per 10,000 births in 2000 was 7.40 (95% CI: 5.33, 10.00) while the most recent statistics from 2014 were 8.48 (6.27, 11.21) **(APPENDIX A)** [2]. Overall, Finland has an incidence of NTDs at 7.4 per 10,000 births and selective terminations. This rate is fairly low and has been relatively stable throughout the years [3].

Much like other parts of the world, diabetes has become a public health concern in Finland. According to the Finnish Diabetes Association, one out of ten people in Finland have diabetes. With a population of over five million, it is estimated that there are about 50,000 people with type 1 diabetes, 250,000 with type 2 diabetes and over 150,000 who remain undiagnosed [4]. In addition, Finland has the highest incidence of type 1 diabetes in the world [5]. With these statistics in mind and because type 2 diabetes continues to rise among women of reproductive age, PGDM continues to be of concern in Finland.

Currently there are only recommendations in Finland regarding folic acid intake, which emphasize intake of folic acid during pregnancy, suggesting a balanced diet rich in folate and 400mg of folic acid for women planning to get pregnant or in their early stages of pregnancy. There are no legislations in Finland regarding mandatory folic acid supplementation in wheat flour, maize flour and rice. Voluntary fortification can be done through approval of the Food Safety Authority. There are, however, many fortified products including cereals, yogurts and juices available to consumers [3, 6].

This study aims to provide insight on the risk of having a child with an NTD among mothers with PGDM using nationwide population-based data in Finland. Since Finland does not currently have policies regarding folic acid fortification, known to reduce the risk of NTDs, estimating the prevalence of NTDs in the country would allow us to determine the need for these mandates.

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

Neural Tube Defects

Neural tube defects (NTDs) are the second most common birth defects occurring globally, following congenital heart defects [7]. NTDs are congenital anomalies of the central nervous system, encompassing the brain and spinal cord, and the bony structures and soft tissue that envelop it [8]. Open neural folds in either the brain or spinal cord prevent adequate covering of these areas due to failed primary neurulation. This can cause an opening to be present that can lead to one of many NTDs including craniorachischisis, anencephaly, and spina bifida [9, 10]. These conditions are serious and often life threatening, the most severe being anencephaly, which always leads to fetal or early neonatal death. Severity can range depending on the location of lesion in spina bifida cases [10].

Etiology of NTDs

Genetic Risk Factors for NTDs

Previous studies have suggested a genetic disposition to NTDs, with an interaction between environmental factors and genetics playing a crucial role in the phenotypic expression of congenital anomalies [11]**.** A small proportion of NTDs is associated with genetic syndromes, including chromosomal anomalies and chromosomal deletion syndromes, while others are associated with single gene mutations. Although there is limited information on the genes associated with non-syndromic NTDs, almost 60-70% of these NTDs are thought to have some sort of

genetic component. Having a family history of NTD increases one's risk of having an NTD, specifically spina bifida and anencephaly [12]. There is also an increased risk of NTDs among those with siblings who have NTDs. Most cases of NTDs tend to have irregular patterns, with various genes involved rather than being caused by a single gene mutation. The various genes involved and the role of environmental factors leads to difficulties in studying genetic risk factors related to NTDs [13]. Research is still limited on identifying genetic variations and susceptibility associated with NTDs. Larger study populations with the use of current methods to analyze multiple genes are needed to better understand the genetic component of this population [12, 13].

Non-genetic Risk Factors for NTDs

A number of non-genetic risk factors are associated with NTDs, especially maternal factors. Obesity is an established risk factor for diabetes, and has been associated with birth defects [14]. There is also an increased risk of birth defects among diabetic women who are obese. In addition, multiplicative interaction affects this risk when obesity and gestational diabetes are both considered, with risk increasing as BMI increases [15]. Since obesity and diabetes involve similar metabolic abnormalities, including insulin resistance and hyperinsulinemia, the increased risk of birth defects among these conditions may have a shared underlying metabolic disorder [16]. Some medications have been found to have adverse effects on a developing embryo. Antiepileptic drugs such as valproic acid decrease folate concentrations in the blood, leading to altered morphogenesis and ultimately NTDs. This is a result of altered folate absorption when taking these medications [17]. Maternal hyperthermia is considered to

be a human teratogen when present during critical periods of prenatal development. One meta-analysis found that generally, elevated core body temperatures through fever or direct exposure to heat posed a greater risk of NTDs. Hyperthermia affects protein synthesis potentially leading to cell death, vascular disruption, membrane disruption or lack of blood supply to the placenta [18].

A critical risk factor of developing NTDs includes deficiency of folate, a vitamin that is often used in its synthetic form, folic acid, for food fortification and supplements. This vitamin is essential during development because of its role in DNA synthesis and protein methylation [19]. It is also critical in regulating homocysteine metabolism, an excess of which occurs from being folate deficient [20]. Folic acid intake during the periconceptional period has been especially important regarding fetal development [21]. Unfortunately, many women are unaware of their pregnancy at this stage. Although folic acid fortification has been found to decrease the risk of spina bifida and anencephaly occurring in children born to women with PGDM, this decreased effect is not present for other birth defects [22]. For diabetic pregnancies, folic acid has been shown to eliminate glucose-induced dysmorphogenesis involved in growth retardation and somatic maldevelopment [23]. Folic acid and other supplements including vitamins C and E, and myo-inositol have antioxidant properties. These properties have been found to offset the adverse outcomes resulting from oxidative stress in the developing fetus in response to maternal hyperglycemia exposure [24]. Overall, folic acid has had prominent effects in preventing NTDs. Women with diabetes that took multivitamins 3 months before pregnancy and during the course of their first trimester had a lower risk

of having children with birth defects [25, 26]. Intake of folic acid in addition to proper glycemic control during preconception decreases the likelihood of diabetes related birth defects [27-29]. While several clinical studies provide evidence of prevention of NTDs through folic acid intake, about 30% of NTDs cannot be prevented through this method [17].

Additionally, vitamin B12 has similar metabolic processes as folate and is important in determining plasma homocysteine as well as producing red blood cells. Low levels of maternal vitamin B12 are associated with an increased risk of having a child with NTDs. Serum holotranscobalamin, used to determine B12 levels, was found to be much lower in mothers who gave birth to a child with an NTD compared to those without NTDs. Another study found the risk of having a child with a NTD was five times greater among women with the lowest levels of vitamin B12 compared to those with the highest levels of vitamin B12 [17, 30, 31]. In a systematic review with over 17 case-control studies, researchers found significantly lower concentrations of B12 in mothers who had children with NTDs compared to a control group [32]. Although a lack of either folate or vitamin B12 can lead to an increased risk of having a child with NTDs, it is not known how the two interact during morphogenesis.

Diabetes Mellitus and Risk of NTDs

It has been well established that maternal diabetes, both PGDM and gestational diabetes (GDM), is associated with an increased risk of NTDs [22, 23, 33]. While the specificities in the mechanism are complex, hyperglycemia has been recognized to play a critical role in these abnormalities during embryogenesis. Mothers with PGDM who

have poor glycemic control, especially during the first trimester, are at greater risk for having a child with a birth defect. The risk of congenital anomalies has been found to be 2 to 11 times higher among children born to mothers with diabetes compared to those without [34]. These malformations have been found to occur similarly among those with both type 1 and type 2 diabetes [15]. Pregnancies involving mothers with PGDM affect nearly 1% of all pregnancies. While this is an issue of concern, unfortunately about a third of women with diabetes are not yet diagnosed [26].

Glucose, an important requirement for oxidative metabolism, is essential to early periods of organogenesis when the embryo needs continuous anaerobic glycolysis prior to placenta development. Early embryos lack pancreatic function until the seventh week of gestation, after the development of β cells. During neural tube closure in the fourth week of gestation, hyperglycemic mothers present an altered in utero environment for the embryo that may lead to improper organogenesis [13].

Glucose intolerance is a common metabolic complication that occurs during pregnancy with 0.2% to 0.3% affected by PGDM [35]. This condition can result in high blood sugar levels or hyperglycemia. Glycosylated hemoglobin (HbA1c) is used to measure average blood sugar levels over a three-month period. This determines the presence of diabetes in those who are undiagnosed or measures adequate glycemic control for those who are diagnosed with diabetes. High levels of maternal HbA1c indicate the extent of hyperglycemia, which is correlated with an increased rate of birth defects and other pregnancy complications in the first trimester of pregnancy [28, 36].

Maternal metabolic abnormalities such as hyperglycemia and increased oxidative stress are an especially critical teratogen when morphogenesis occurs, leading to changes in embryonic development [28]. Diabetes-induced hypoxia, which is a lack of oxygen spreading to tissues, may also be another mechanism to consider during abnormal embryonic development. Overall, the mechanisms of diabetes-induced neural tube defects are not clear [28, 37-39].

Increases in maternal glucose concentrations lead to elevated levels of reactive oxygen species (ROS), which are essential for cell signaling and homeostasis. This may result in damaged cell structures and oxidative stress for the embryo and fetus. Elevated ROS levels decrease the natural antioxidant capacity of fetal cells, causing birth defects through membrane alterations, mitochondrial dysfunction and initiation of apoptosis - abnormal programmed cell death [24, 28, 40, 41]. Furthermore, regarding mitochondrial dysfunction, increased maternal glucose levels initiate excess oxidative phosphorylation in the mitochondria. This leads to an accumulation of ROS and eventually damages fetal tissue and cells [28, 42].

PGDM may affect the regulation of gene expression by altering the embryonic epigenome leading to abnormal morphogenesis. Experiments examining global gene expression show that PGDM has a considerable impact on the transcriptional profile of embryos exposed to diabetes compared to those unexposed [43-45]. It is important to consider that there is variation in embryopathy among offspring exposed to diabetes depending on whether a defect occurs or even the extent of the defect. PGDM leads to greater variability among gene expression in embryos exposed to diabetes. This may

result in incomplete phenotype penetrance, having a mutation in a specific gene but not displaying features of the disorder or condition. This suggests an alternative perspective to the idea of a common response pathway, one where the presences of underlying differences between individuals determine the outcomes of development [28, 45]. A study conducted by Kappen and Salbaum (2014) investigated the molecular differences among those exposed to maternal hyperglycemia compared to those unexposed. The findings propose a greater extent of genetic variability among those exposed, leading to decreased precision in gene regulation among this population that may be caused from abnormal epigenetic regulation. While specific mechanisms are still unknown, focusing on genes with variation among individuals can be targeted to determine how these genes are altered [46].

PGDM affects epigenetic regulation causing an impact on mechanisms involving DNA methylation and histone acetylation, essential components of gene expression and regulation in the developing embryo [28, 44, 47-49]. These alterations have an impact on genes involved in morphogenetic processes that include neural tube closure or heart development. In turn, studies have demonstrated that neural tube defects and congenital heart defects are among the most common congenital anomalies among diabetic pregnancies [28, 44, 45]. While previous studies have been instrumental in examining genetic factors of the development of structural abnormalities, upstream regulatory mechanisms that alter gene expression have yet to be discovered. Therefore, it is proposed that the presence of maternal hyperglycemia affects several epigenetic levels in regards to gene regulation [28].

Based on previous studies on mice, outcomes among pregnancies affected by PGDM of outbred mice were a result of genetic differences whereas those of inbred mice were more likely to be non-genetic [50]. This led to the idea that abnormalities resulting from diabetic pregnancies in humans were regulated at the epigenetic level by mechanisms involving DNA methylation, chromatin modification, transcriptional regulation and oxidative stress. Studies on mice with genetically and environmentally induced diabetes demonstrate that maternal hyperglycemia can activate abnormal cell death signaling in a developing embryo leading to malformations and even death [28, 51]. Other animal studies have determined that even with diabetes in the mother, blocking cell death pathways can reduce the amount of malformed embryos resulting from hyperglycemic conditions [28, 52, 53].

Neural tube defects (NTDs) are life-threatening defects that occur in the brain and spine early in pregnancy. While there are several risk factors for NTDs, maternal hyperglycemia is a major concern due to the increased prevalence of diabetes among women of reproductive age. The literature provided suggest how detrimental exposure of maternal hyperglycemia can be for the development of an embryo. It is also important to consider the preventative effects of folic acid in combatting the adverse effects maternal diabetes has on the pregnancy. This issue is especially important in Europe, where folic acid fortification has still not been mandated. This study aims to provide insight on the risk of having a child with an NTD among mothers with PGDM using nationwide population-based data in Finland. Since Finland does not currently have policies regarding folic acid fortification, known to reduce the risk of NTDs,

estimating the prevalence of NTDs in the country would allow us to determine the need for these mandates. This is especially needed among women with PGDM who are pregnant or are planning to get pregnant as Finland has the highest prevalence of type 1 diabetes in the world [54].

CHAPTER 3

METHODS

Data Sources

We used data from multiple nationwide healthcare registries in Finland that were linked using unique identity codes for the mother and newborn, including all citizens and permanent residents of Finland.

The Finnish Hospital Discharge Register (FHDR) was used to obtain information on inpatient care in hospitals and primary health care centers [55]. This database includes nationwide linkable data on all hospital discharges and identification codes. Data for each record includes several variables, some of which are date of birth, sex, area of residence, hospital ID, admission and discharge days, patient diagnosis and surgical procedures. Diagnoses codes are coded using the International Classification of Diseases and Related Health Problems –classification system's 10th version (ICD-10) since 1996. As on of the first individual level hospital discharge registers in the world, FHDR has been widely used in previous research. Information from FHDR has been found to be complete, as well as both accurate and valid [56].

Finnish Medical Birth Register (MBR) was used to access data on maternal demographic and health data [57]. The MBR, maintained by the National Institute for Health and Welfare, provided information on live birth and stillbirth data since 1987. Data from the MBR is used to develop and organize maternity care, obstetric services and neonatal care. Comprehensive information on the mother, infant and delivery are also recorded [57]. Research on the quality of data obtained from this registry found

that the data was generally valid and is actively being improved due to its continuous use in research [58, 59].

The Register of Congenital Malformations (RCM) was used to access data on congenital chromosomal and structural anomalies found in live births and stillbirths in fetuses throughout Finland [60]. Information on congenital anomalies includes verbal diagnosis, ICD-10 codes, pattern of anomaly (isolated, multiple anomalies, syndrome), time of detection and a variety of other details [60]. Although the RCM includes information on terminations, the diagnoses are not collected systematically and therefore terminations could not be considered for this study.

Census data from Statistics Finland was also used for population-level information on income and education [61]. The data sources involving individual-level data are not available publicly. The linkages were done through personal identifiers and the final dataset used for this study was anonymized without these identifiers.

Study Population

The study included all live births and stillbirths in Finland from 2000-2014, comprising of 876,912 births. Data on elective terminations were not available during the study period, and hence cases that resulted in elective terminations were not included in the analysis. In Finland, the proportion of terminations of pregnancy for fetal anomaly (TOPFA) due to NTDs between years 2000-2014 was 70.4% (95% CI: 67.1, 73.4) [2]. Although case definition criterion included stillbirths, there were no stillbirths among those with NTDs during our study years. Records were linked using the child's unique identity code with maternal identification (ID) codes. Linkages are made for all

women with valid ID codes, which account for 99.8% of women in Finland who are given these codes. The average percentage of subjects that were successfully linked between all data sources during our study period was 99.79%.

NTD Cases

NTD diagnosis was established using the RCM where diagnoses of congenital anomalies were determined at birth using ICD-10 codes. Cases included births with at least one neural tube defect. ICD-10 codes between 740020 and 740100 were considered as anencephaly, including craniorachischisis. Codes from 741000 to 741999 denoted spina bifida and codes from 742000 to 742090 described encephalocele, including cranial meningocele and encephalomyelocele. When looking specifically at concurring birth defects, we included isolated and multiple anomalies among NTD cases. Congenital anomalies associated with genetic syndromes were not included.

Controls

Controls for this study include infants that were recorded as live births or stillbirths and with no major congenital malformations. Controls could have minor congenital anomalies.

Exposure Variable

The MBR provides detailed information including maternal diagnoses during pregnancy and delivery. Diagnosis of diabetes, the main exposure variable, was established using ICD-10 codes. This was created with codes O24.0-O24.3 that refer to pre-existing diabetes mellitus or PGDM (insulin-dependent, non-insulin dependent, malnutrition-related and unspecified).

Co-Variables

Multiple co-variables were selected based on *a priori* criteria through a thorough review of previous studies. Co-variables included maternal age at delivery (<20, 20-24, 25-29, 30-34, 35 years or more); household income level (<20th percentile, 20-80th percentile, >80th percentile); maternal education (No education or basic education, upper secondary or pre-bachelors education and bachelors or greater); maternal body mass index (kg/m^2) (<18.5, 18.5-24.9, 25-29.9, 30 or more); whether or not the child was born preterm (gestational age <37 weeks); child birth weight (<1500g, 1500-2499g, 2500-3499g, 3500-4500g). Maternal health variables were examined for the following diagnoses: gestational diabetes using ICD-10 codes O24.4 and O24.9 relating to diabetes mellitus arising in pregnancy and diabetes in pregnancy that is unspecified; pre-existing hypertension using ICD-10 codes O10.0-O10.4, O10.9 for unspecified pre-existing hypertension, O11 for pre-existing hypertensive disorder, and gestational hypertension using ICD-10 code O13 to identify women with gestational hypertension without significant proteinuria.

Statistical Analysis

Descriptive analyses were conducted to compare infant and maternal characteristics for cases and controls. Frequencies and percentages were estimated, and comparisons were conducted using chi square tests. We used logistic regression analysis to estimate the crude odds ratios (cORs) and 95% confidence intervals (CIs) to examine the association between selected infant and maternal characteristics and NTD outcome. Analyses were stratified by isolated and multiple cases of NTDs. Multivariate

logistic regression was used to estimate adjusted ORs (aORs) and 95% CIs, adjusting for co-variables selected on *a priori* basis (i.e., maternal age categories, household income percentile, education level, maternal BMI and child's birth weight). All analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC). The Emory University Institutional Review Board has determined that the present study does not require IRB review as it was based on secondary de-identified data.

CHAPTER 4

RESULTS

The study examined a total of 876,912 live births and stillbirths that occurred between 2000 and 2014 in Finland. Of these, 240 were identified as NTD cases with or without other major congenital abnormalities. The remaining live births and stillbirths, without NTDs were considered to be controls in our analysis (n=876,672). Of the 240 cases, 144 were determined as isolated NTD cases and the remaining cases had other multiple major congenital anomalies. In addition, no stillbirths were seen among cases of NTDs.

Overall, 4,112 live or stillbirths (0.47%) were identified among mothers with PGDM. PGDM was reported in 1.67% of case mothers and 0.47% of control mothers. In our unadjusted analysis, we did not find a significant association between maternal PGDM and the risk of any NTD (cOR=3.60; 95% CI: 0.97, 9.35) or an isolated NTD (cOR=2.99; 95% CI: 0.36, 11.01) **(Table 1).** Other co-variables that were significantly associated with an increased risk of any NTD in the unadjusted analysis include: income being greater than 80th percentile (cOR= 1.77; 95% CI: 1.22, 2.56), maternal BMI 30 or more (cOR=1.67; 95% CI: 1.03, 2.63), child being preterm (cOR= 4.80; 95% CI: 3.55, 6.49), birth weight <1500 g (cOR=6.10; 95% CI: 3.43, 10.85), birth weight between 1500-2499 g (cOR=4.73; 95% CI: 3.32, 6.73) and having multiple major congenital anomalies (cOR=4.65; 95% CI: 3.48, 6.22). For isolated NTDs specifically, the following maternal and child characteristics were significantly associated with increased odds of NTDs:

pre-pregnancy BMI 30 or more (cOR=2.31; 95% CI: 1.27, 4.04) and child being preterm (cOR= 2.02; 95% CI: 1.20, 3.40) **(Table 1).**

We examined unadjusted association between maternal and child characteristics and PGDM. Mother's weight was significantly associated with PGDM (cOR for BMI category 25-29.9 vs. 18.5-24.9=1.83; 95% CI: 1.70, 1.97). Similarly, maternal obesity (BMI=30 or more) was associated with PGDM (cOR=3.43; 95% CI: 3.18, 3.70). Women who were underweight with a BMI <18.5 had a decreased likelihood of being having PGDM (cOR=0.62; 95% CI: 0.48, 0.79). Pre-existing hypertension was significantly associated with an increased likelihood of PGDM (cOR=8.33; 95% CI: 7.23, 9.61). Those with gestational hypertension were 3.14 (95% CI: 2.75, 3.58) times likely to have PGDM compared to those without. Other factors associated with having PGDM include preterm birth (cOR= 7.78; 95% CI: 7.28, 8.31), child's birth weight being <1500 g (cOR=2.55; 95% CI: 1.98, 3.29) and child's birth weight between 1500-2499 g (cOR= 2.13; 95% CI: 1.85, 2.46) **(Table 2).**

We conducted multivariable analyses to examine the association between PGDM and NTDs, controlling for maternal age, income, education, maternal BMI and child's birth weight. After controlling for aforementioned co-variables, we found that mothers with PGDM were 3.25 (95% CI: 1.03, 10.27) times more likely to have a child with any NTD compared to mothers without PGDM, which was a significant positive association. When examining the same association restricting to isolated NTDs only, we noted the positive effect, but the confidence interval included the null (aOR=3.34; 95% CI: 0.82, 13.69) **(Table 3)**.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Our large national registry-based population-representative case-control study in Finland examined 876,912 live and stillbirths during years 2000-2014. We found a three-fold increased risk of NTD in the offspring among mothers with PGDM compared to those without. However, our findings are based on live births only. There were no stillbirths among cases of NTDs during the study period. Our study provides evidence that PGDM is significantly associated with a risk of having a child with any NTD; however, the association needs to be further examined considering all birth outcomes (i.e., elective terminations and early fetal losses) to have a complete knowledge of the etiology association with PGDM.

Our findings are consistent with previous studies reporting that less than 1% of pregnancies are affected by PGDM [23]. Additionally, our study findings are similar to previous studies demonstrating that PGDM leads to more than a two-fold increase in risk of having a child with a NTD [23, 26]. The prevalence of diabetes in Nordic countries, which include Denmark, Norway, Sweden, Finland and Iceland, has been found to be similar [62]. A Finnish population-based cohort study examined 649,043 live births between 2004 and 2014. The results determined that 0.62% of births were affected by type 1 diabetes while 0.57% of births were affected by type 2 diabetes [63]. In a Denmark national cohort study including all singleton live births between 1978 and 2011, 2,025,727 births were identified. Among these births, 7296 (0.36%) were exposed to maternal PGDM [64]. In a study examining maternal diabetes in Norway, researchers

identified 914,427 singleton births, of which 0.61% were affected by PGDM [65]. The variations in these studies could be due to lack of information on all pregnancy outcomes, including terminations, which may underestimate rates of NTDs.

Our data is consistent with studies that have found an association between PGDM and risk of having a child with NTDs. We found that women with PGDM are 3.25 times more likely to have a child with an NTD. Correa et al. (2008) previously found that this association was 3.39 (95% CI: 1.11, 10.31) times greater for those with anencephaly and 2.09 (95% CI: 0.26, 16.56) times greater for those with encephalocele while the relationship for spina bifida was not as great (OR=0.75; 95% CI: 0.17, 3.24) [22]. Another study conducted in Hungary found that mothers with type 1 diabetes were 1.1 (95% CI: 0.3, 3.4) times more likely to have a child with an isolated NTD while mothers with type 2 diabetes were also 1.1 (0.5, 2.8) times more likely to have a child with an isolated NTD compared to controls [66]. A study considering non-genetic risk factors for NTDs using population-based data found that births exposed to PGDM were 1.78 (95% CI: 0.6, 2.46) times more likely to have anencephaly. This study did not find a significant association with other NTDs [14].

According to our findings, mothers with a BMI of 30 or more have a greater risk of having a child with an NTD, this association is even greater in regards to isolated NTDs. Increased BMI and obesity has largely been considered a risk factor for NTDs [15, 67, 68]. Obesity has been found to be closely associated with risk of NTD, and may modify the association between diabetes are NTDs [15, 69, 70]. We found that those with a pre-pregnancy BMI of 30 and more are also at an increased risk of having a child

with a NTD. Further studies should consider the interaction between diabetes and obesity among this Finnish population.

While the current study does not include information on terminations, it is important to consider all pregnancy outcomes. EUROCAT surveillance data from 2011-2015 demonstrated that 76% of pregnancies that were affected by NTDs resulted in elective terminations, with roughly 20% resulting in live births [2]. One study utilizing EUROCAT registries form 19 European countries between 1991 and 2011 found that the pooled prevalence throughout the study period was 9.1 per 10,000 births. When looking specifically at Finland, they found this rate to be 8.67 per 10,000 births, which is almost three times higher than the prevalence we found. Therefore, it is important to include all outcomes of pregnancy in order to prevent underestimation and biased results [71].

Although we found a significant association between any NTD and PGDM, this relationship was not significant among isolated cases of NTDs. One study using the National Birth Defects Prevention Study (NBDPS) looked at PGDM in both isolated and multiple cases of birth defects. This study found that the association between PGDM was almost three times higher among those with multiple defects (aOR=7.80; 95% CI: 4.66, 13.05) compared to those with isolated birth defects (aOR=2.34; 95% CI: 1.44, 3.81) [22]. Our study found that those with multiple major congenital anomalies were over four times more likely to have a NTD than those with isolated congenital anomalies (cOR=4.65; 95% CI: 3.48, 6.22).

Strengths of this study include nationally-representative, population-based datasets on births linking multiple data sources to study various infant and maternal

characteristics for the association between PGDM and NTDs. The time period for the study covered a span of fifteen years. The study data comes from high quality data registries found to be both valid and reliable. The completeness of data and validity of linkages of these registers have been well established, especially since they have been widely used for epidemiological studies [56, 58, 59, 72, 73]. Since each permanent resident in Finland is given a unique identification number used to link between various data sources, linkage errors are minimized [56]. Finnish registers employ standardized procedures to define NTDs and PGDM using ICD-10 codes. Finland, among other Nordic countries, has a comprehensive population-based medical birth register that details information on new births. In addition, registries on congenital anomalies include information on children until one year of age [57, 74]. This study allows us to address the current association between PGDM and NTDs in Finland, which is comparable to other Nordic countries.

There are some important limitations to our study. Data on elective terminations were not available for this study, which leads to underestimations of NTDs. Another limitation of this study is lack of information on vitamin and supplement intake of mothers during our study period. The MBR recently implemented data collection on folic acid supplementation in 2017, which occurred after our study period. Information on folic acid is important, as it is known to reduce the risk of having a child with an NTD. This could have shed light on whether or not there was modification among folic acid use on women with PGDM. In addition, information on glucose control would also have allowed us to test for interaction.

Stillbirths are common among NTDs, especially anencephaly [8]. We are interested in understanding whether or not the risk of PGDM or NTDs differs among live births compared to still births. Considering all EUROCAT countries that are full members, the proportion of fetal death from 20 weeks of gestation among NTDs decreased from 4.7% (95% CI: 3.2, 6.9) in 2000 to 3.7% (95% CI: 2.5, 5.5) in 2014. In addition, there was a combined proportion of 4.5% (95% CI: 4.1, 4.9) fetal deaths among NTDs throughout the years 2000-2014. When looking specifically at Finland, whose criteria involve fetal death from 22 weeks of gestation, this proportion was 2.4% (95% CI: 0.4, 12.3) in 2000 and 2.0% (95% CI: 0.4, 10.7) in 2014. The combined proportion of fetal deaths due to NTDs in Finland from 2000-2014 was 2.5% (95% CI: 1.7, 3.9) [2]. There were no stillbirths among the cases in our population, possibly due to the high rates of elective terminations. However, data on stillbirth allow for a better comparison of the effect of PGDM on NTDs.

The 2007 FINDIET Survey is conducted every five years to report on diet and nutrient intake of the Finnish population. This survey found that the average intake of folate fell below recommended levels for women aged 24-64 years old [75]. A large surveillance study using EUROCAT data including over 11,000 cases of NTDs across 19 European countries from 1991 to 2011 suggested preconceptional folic acid intake and voluntary supplementation have not been effective in reducing prevalence rates of NTDs in Europe within the last few decades [71]. One review assessing the efficacy of folic acid food fortification programs in reducing the risk of NTDs found that the estimated prevalence of NTDs that is reasonable with adequate folic acid intake is about

5-6 cases per 10,000 pregnancies [19]. There may be other factors to consider in terms of preventing NTDs such as the amount of pregnancies planned which can range from 37-86% in Finland. In addition, on average, the first prenatal visit in Finland occurs at 9 weeks. This is several weeks after prevention of NTDs can occur since neurulation occurs by 4 weeks of gestation [3].

Women with pregestational diabetes are at an increased risk of having a child with birth defects; especially NTDs. Finland has the highest incidence of type 1 diabetes in the world, with about 1 in 200 children under the age of 15 being affected. More than 500,000 of the 5.5 million people in Finland have diabetes, 75% of those being diagnosed with type 2 diabetes [54]. As the prevalence of diabetes continues to increase, prevention of birth defects in offspring among women with PGDM is important to consider. In Finland, there is currently no mandatory folic acid food fortification. Finland, among a few other European countries, considers no need for folic acid fortification if a proper diet is in play. Their low rates of NTDs might be an indicator of the high rates of terminations among affected pregnancies. This could also be associated with proper glucose control before and during early pregnancy, which is associated with a lower risk of birth defects. In Finland, and other countries lacking folic acid intervention, women should be highly encouraged to consume folic acid supplements, especially those of reproductive age or planning to get pregnant.

Overall, our study showed there was a significant association between PGDM and NTDs risk among live births in Finland. There were no stillborn cases in our study. Including information from cases that were electively terminated may provide a better

insight into this association in the future. Our study adds to previous literature that underscores the effect of PGDM on NTDs. This study aims to increase awareness on preventative measures for both PGDM and NTDs, like proper glucose control during pregnancy, especially for women with PGDM, and folic acid fortification of extensively consumed staple foods like wheat and maize products. These findings suggest that PGDM should be monitored with treatments implemented periconceptionally. Further studies are required to better understand this association using all pregnancy outcomes in Finland. Additionally, future studies should consider how folic acid and proper glucose control might affect the association between PGDM and NTDs in Finland.

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Table 1. Maternal and Infant Characteristics for Neural Tube Defects: Cases and Controls in Finland, 2000-2014

	Controls (n=876672)	All Cases[a] (n=240)	Crude OR (95% CI)	Isolated NTDs (n=144)	Crude OR (95% CI)
	n (%)	n (%)		n (%)	
Maternal Characteristics					
Maternal Age					
<20 years	22074 (2.52)	9 (3.75)	1.53 (0.78, 2.98)	6 (4.17)	1.69 (0.74, 3.83)
20-34 years	688008 (78.48)	184 (76.67)	REFERENT	111 (77.08)	REFERENT
≥35 years	166590 (19.00)	47 (19.58)	1.06 (0.77, 1.45)	27 (18.75)	1.01 (0.66, 1.53)
Income					
<20th percentile	77098 (8.79)	26 (10.83)	1.35 (0.89, 2.04)	14 (9.72)	1.15 (0.66, 2.01)
20-80th percentile	606664 (69.20)	152 (63.33)	REFERENT	96 (66.67)	REFERENT
>80th percentile	76836 (8.76)	34 (14.17)	1.77 (1.22, 2.56)	17 (11.81)	1.40 (0.84, 2.34)
Nativity					
Finnish background, born in Finland	795004 (90.68)	209 (87.08)	REFERENT	127 (88.19)	REFERENT
Other	73727 (8.41)	28 (11.67)	1.45 (0.97, 2.14)	15 (10.42)	1.27 (0.75, 2.18)
Education					
No education or basic	378506 (43.18)	105 (43.75)	1.05 (0.80, 1.37)	65 (45.14)	1.10 (0.78, 1.56)
Upper secondary - Pre-Bachelors	403502 (46.03)	107 (44.58)	REFERENT	63 (43.75)	REFERENT
Bachelors or greater	94664 (10.80)	28 (11.67)	1.12 (0.74, 1.69)	16 (11.11)	1.08 (0.63, 1.87)
BMI (kg/m^2)[b, c]					
<18.5	23165 (2.64)	2 (0.83)	0.41 (0.05, 1.53)	2 (1.39)	0.77 (0.09, 2.97)
18.5-24.9	385521 (43.98)	81 (33.75)	REFERENT	43 (29.86)	REFERENT
25-29.9	134930 (15.39)	34 (14.17)	1.20 (0.78, 1.81)	23 (15.97)	1.53 (0.88, 2.59)
≥30	73913 (8.43)	26 (10.83)	1.67 (1.03, 2.63)	19 (13.19)	2.31 (1.27, 4.04)
Pregestational Diabetes[b, c]					
No	872564 (99.53)	236 (98.33)	REFERENT	142 (98.61)	REFERENT
Yes	4108 (0.47)	4 (1.67)	3.60 (0.97, 9.35)	2 (1.39)	2.99 (0.36, 11.01)
Gestational Diabetes- 1st Trimester					
No	825519 (94.17)	230 (95.83)	REFERENT	137 (95.14)	REFERENT
Yes	51153 (5.83)	10 (4.17)	0.70 (0.37, 1.32)	7 (4.86)	0.83 (0.39, 1.76)
Smoking Status					
Never Smoked	722785 (82.45)	198 (82.50)	REFERENT	122 (84.72)	REFERENT
Stopped during 1st trimester	38095 (4.35)	10 (4.17)	0.96 (0.51, 1.81)	6 (4.17)	0.93 (0.41, 2.12)
Continued after 1st trimester	93605 (10.68)	22 (9.17)	0.86 (0.55, 1.33)	10 (6.94)	0.63 (0.33, 1.21)
Pre-existing Hypertension[b, c]					
No	870978 (99.35)	239 (99.58)	REFERENT	143 (99.31)	REFERENT
Yes	5694 (0.65)	1 (0.42)	0.64 (0.02, 3.60)	1 (0.69)	1.07 (0.03, 6.06)

Gestational Hypertension[b, c]					
No	859963 (98.09)	237 (98.75)	REFERENT	141 (97.92)	REFERENT
Yes	16709 (1.91)	3 (1.25)	0.65 (0.13, 1.93)	3 (2.08)	1.20 (0.22, 3.27)
Parity					
0 Previous Births	365255 (41.66)	88 (36.67)	REFERENT	55 (38.19)	REFERENT
1 or more Previous Births	510671 (58.25)	152 (63.33)	0.81 (0.62, 1.05)	89 (61.81)	0.86 (0.62, 1.21)
Child Characteristics					
Child Sex					
Male	448535 (51.16)	127 (52.92)	REFERENT	79 (54.86)	REFERENT
Female	428098 (48.83)	113 (47.08)	0.93 (0.72, 1.20)	65 (45.14)	0.86 (0.62, 1.20)
Preterm (or Gestational Age)					
Not Preterm; ≥37 weeks	823153 (93.90)	185 (77.08)	REFERENT	128 (88.89)	REFERENT
Preterm; <37 weeks	50963 (5.81)	55 (22.92)	4.80 (3.55, 6.49)	16 (11.11)	2.02 (1.20, 3.40)
Child Birthweight (g)[c]					
<1500	7296 (0.83)	13 (5.42)	6.10 (3.43, 10.85)	2 (1.39)	1.46 (0.36, 5.96)
1500-2499	31153 (3.55)	43 (17.92)	4.73 (3.32, 6.73)	11 (7.64)	1.88 (1.00, 3.56)
2500-3499	373379 (42.59)	109 (45.42)	REFERENT	70 (48.61)	REFERENT
3500-4500	441029 (50.31)	67 (27.92)	0.52 (0.38, 0.71)	57 (39.58)	0.70 (0.49, 0.98)
Concurring Birth Defect (Pattern)					
Isolated Congenital Anomaly	28916 (3.30)	144 (60.00)	REFERENT	NA	NA
Multiple Major Congenital Anomalies	2936 (0.33)	68 (28.33)	4.65 (3.48, 6.22)	NA	NA

[a] Cases include children born with anencephaly, spina bifida, encephalocele
[b] exact odds ratios were used for crude association between all NTDs and controls
[c] exact odds ratios were used for crude association between all NTDs and controls

Table 2. Maternal and Infant Characteristics and Maternal Pregestational Diabetes Mellitus (PGDM) in Finland, 2000-2014

	No Pre-gestational DM (n=872800)	**Pre-gestational DM (n=4112)**	**Crude ORs (95% CI)**
	n (%)	n (%)	
Maternal Characteristics			
Maternal Age			
<20 years	22002 (2.52)	81 (1.97)	0.83 (0.66, 1.03)
20-34 years	685142 (78.50)	3050 (74.17)	REFERENT
≥35 years	165656 (18.98)	981 (23.86)	1.33 (1.24, 1.43)
Income			
<20th percentile	76711 (8.79)	413 (10.04)	1.14 (1.03, 1.26)
20-80th percentile	603958 (69.20)	2858 (69.50)	REFERENT
>80th percentile	76549 (8.77)	321 (7.81)	0.89 (0.80, 1.00)
Nativity			
Finnish background, born in Finland	791389 (90.67)	3824 (93.00)	REFERENT
Other	73472 (8.42)	283 (6.88)	0.80 (0.71, 0.90)
Education			
No education or basic	376499 (43.14)	2112 (51.36)	1.31 (1.23, 1.40)
Upper secondary - Pre-Bachelors	401892 (46.05)	1717 (41.76)	REFERENT
Bachelors or greater	94409 (10.82)	283 (6.88)	0.70 (0.62, 0.80)
BMI (kg/m^2)			
<18.5	23102 (2.65)	65 (1.58)	0.62 (0.48, 0.79)
18.5-24.9	383853 (43.98)	1749 (42.53)	REFERENT
25-29.9	133849 (15.34)	1115 (27.12)	1.83 (1.70, 1.97)
≥30	72802 (8.34)	1137 (27.65)	3.43 (3.18, 3.70)
Pregestational Diabetes			
No	NA	NA	NA
Yes	NA	NA	NA
Gestational Diabetes- 1st Trimester			
No	NA	NA	NA
Yes	NA	NA	NA
Smoking Status			
Never Smoked	719679 (82.46)	3304 (80.35)	REFERENT
Stopped during 1st trimester	37845 (4.34)	260 (6.32)	1.50 (1.32, 1.70)
Continued after 1st trimester	93162 (10.67)	465 (11.31)	1.09 (0.99, 1.20)

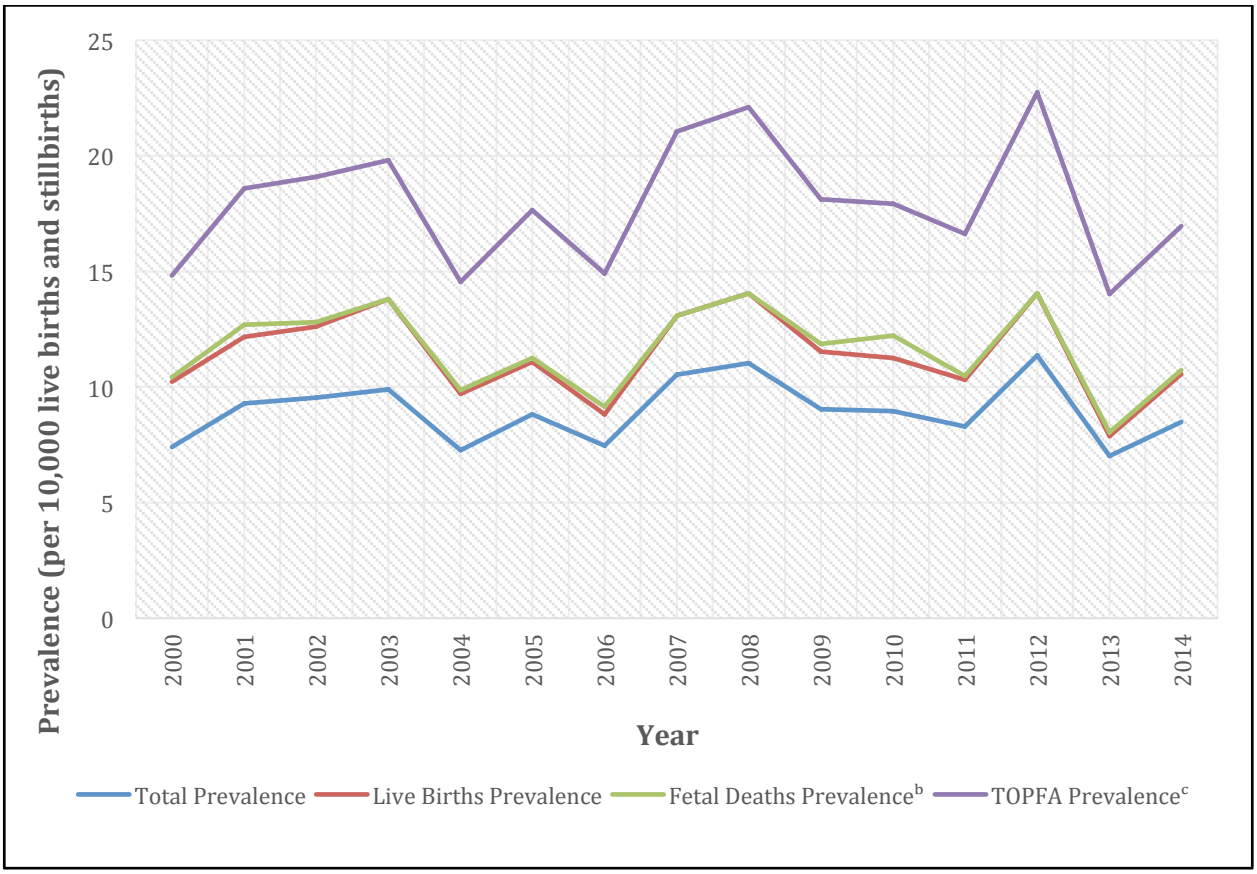
Pre-existing Hypertension			
No	867311 (99.37)	3906 (94.99)	REFERENT
Yes	5489 (0.63)	206 (5.01)	8.33 (7.23, 9.61)
Gestational Hypertension			
No	856322 (98.11)	3878 (94.31)	REFERENT
Yes	16478 (1.89)	234 (5.69)	3.14 (2.75, 3.58)
Parity			
0 Previous Births	363607 (41.66)	1736 (42.22)	REFERENT
1 or More Previous Births	508447 (58.25)	2376 (57.78)	1.02 (0.96, 1.09)
Child Characteristics			
Child Sex			
Male	446584 (51.17)	2078 (50.54)	REFERENT
Female	426177 (48.83)	2034 (49.46)	1.03 (0.97, 1.09)
Preterm (or Gestational Age)			
Not Preterm; ≥37 weeks	820548 (94.01)	2790 (67.85)	REFERENT
Preterm; <37 weeks	49704 (5.69)	1314 (31.96)	7.78 (7.28, 8.31)
Child Birthweight (g)			
<1500	7246 (0.83)	63 (1.53)	2.55 (1.98, 3.29)
1500-2499	30971 (3.55)	225 (5.47)	2.13 (1.85, 2.46)
2500-3499	372218 (42.65)	1270 (30.89)	REFERENT
3500-4500	438867 (50.28)	2229 (54.21)	1.49 (1.40, 1.60)
Concurring Birth Defect (Pattern)			
Isolated Congenital Anomaly	28748 (3.29)	312 (7.59)	REFERENT
Multiple Major Congenital Anomalies	2959 (0.34)	45 (1.09)	1.40 (1.02, 1.92)

Table 3. Adjusted Analysis for the Association between Pregestational Diabetes Mellitus (PGDM) and Neural Tube Defects in Finland, 2000-2014

Characteristic		Adjusted (All NTDs)	Adjusted (Isolated NTDs)
Pregestational Diabetes			
	No	REFERENT	REFERENT
	Yes	3.25 (1.03, 10.27)	3.34 (0.82, 13.69)
Maternal Age			
	<20 years	1.02 (0.32, 3.26)	1.10 (0.26, 4.59)
	20-34 years	REFERENT	REFERENT
	≥35 years	0.86 (0.53, 1.41)	1.14 (0.63, 2.07)
Income			
	<20th percentile	1.33 (0.78, 2.28)	1.30 (0.66, 2.55)
	20-80th percentile	REFERENT	REFERENT
	>80th percentile	1.83 (1.10, 3.05)	1.50 (0.75, 3.00)
Education			
	No education or basic	1.10 (0.74, 1.62)	1.34 (0.82, 2.20)
	Upper secondary - Pre-Bachelors	REFERENT	REFERENT
	Bachelors or greater	1.05 (0.55, 2.03)	1.15 (0.50, 2.64)
BMI (kg/m^2)[b]			
	<18.5	0.42 (0.10, 1.70)	0.85 (0.20, 3.53)
	18.5-24.9	REFERENT	REFERENT
	25-29.9	1.35 (0.88, 2.06)	1.74 (1.03, 2.95)
	≥30	1.72 (1.06, 2.79)	2.41 (1.03, 2.95)
Child Birthweight (g)			
	<1500	5.25 (2.26, 12.18)	2.71 (0.65, 11.29)
	1500-2499	4.40 (2.69, 7.17)	1.91 (0.81, 4.54)
	2500-3499	REFERENT	REFERENT
	3500-4500	0.500 (0.33, 0.75)	0.71 (0.44, 1.13)

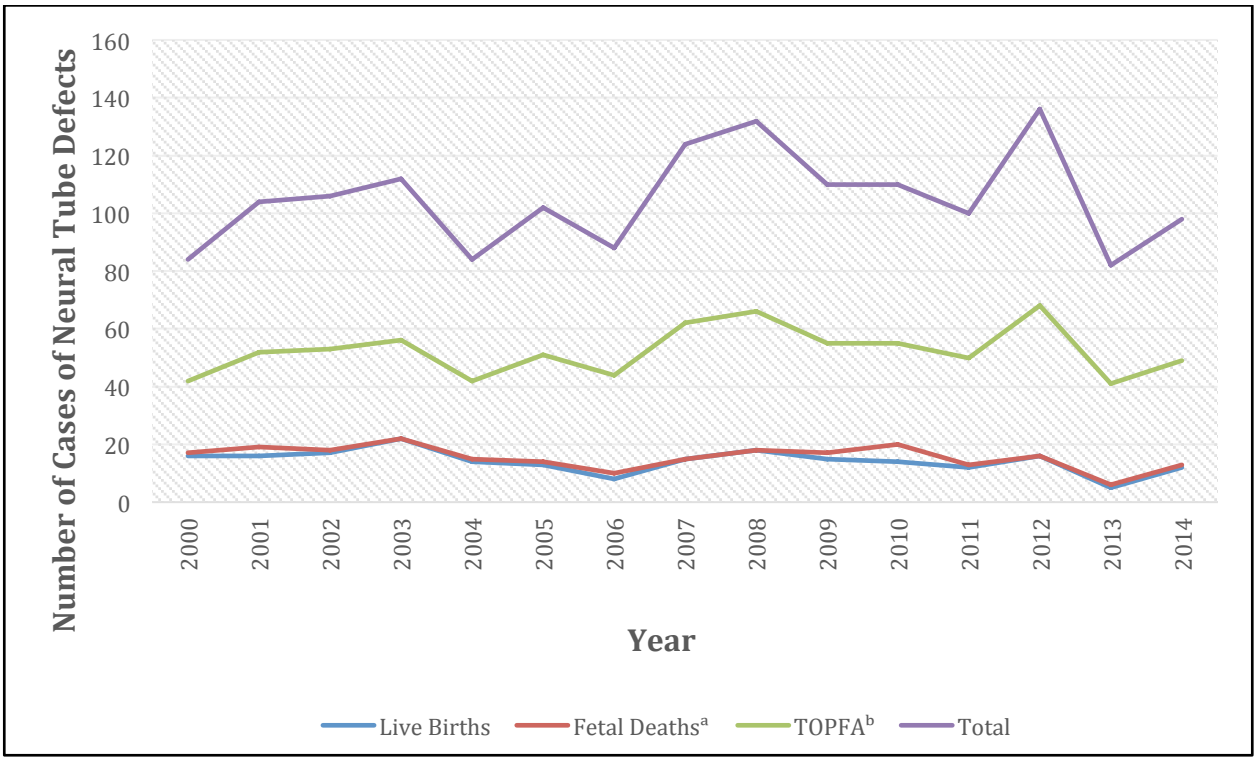
Each variable was adjusted for all other variables in the model

APPENDIX A. Prevalence per 10,000 Live Births and Stillbirths of Neural Tube Defects in Finland, 2000-2014 using data obtained from EUROCAT[a]



[a]Prevalence is calculated as a ratio of number of cases in livebirths, fetal deaths and TOPFA among all live births and stillbirths per 10,000 live births and still births in Finland
[b]Fetal deaths and stillbirths are considered from 22 weeks gestation in Finland
[c]TOPFA: Termination of pregnancy for fetal anomaly following prenatal diagnosis

APPENDIX B. Number of Cases of Neural Tube Defects in Finland, 2000-2014 using data obtained from EUROCAT



[a]Fetal deaths and stillbirths are considered from 22 weeks gestation in Finland
[b]TOPFA: Termination of pregnancy for fetal anomaly following prenatal diagnosis