Congenital heart defects in Norway – a nation-wide study of birth prevalence, maternal diabetes, and folic acid supplementation in pregnancy

Elisabeth Leirgul

Dissertation for the degree of philosophiae doctor (PhD)
at the University of Bergen

2016

Dissertation date: 24.05.2016

© Copyright Elisabeth Leirgul

The material in this publication is protected by copyright law.

Year: 2016

Title: Congenital heart defects in Norway- a nation-wide study of birth prevalence, maternal diabetes, and folic acid supplementation in pregnancy

Author: Elisabeth Leirgul

Print: AiT Bjerch AS / University og Bergen

Scientific environment

Genetic Epidemiology research group, Department of Global Public Health and Primary Care
University of Bergen, Norway

The study was funded by Research Council Norway and the Western Norway Regional Health Authority.

Acknowledgements

The present work was carried out during the period 2011-2015 at the Department of Global Public Health and Primary Care at the University of Bergen and was funded by Research Council Norway.

I have had the privilege of having three highly experienced researchers with different areas of expertise supervising the work. First of all I want to thank my main supervisor, Nina Øyen, for introducing me to the scientific field of epidemiology and being a steadfast teacher through the years. I am grateful for her dedication to the project; for tirelessly challenging my scientific approaches, her invaluable attention to details, and continuously strive for perfection. Gottfried Greve has been my nearest supervisor at the Department of Heart Disease at Haukeland University Hospital for more than a decade, and also a co-supervisor at this project. I am thankful for his loyal support and encouragement, generous availability, wise advises, and valuable academic contributions. My thanks also goes to my second co-supervisor, Stein Emil Vollset for his sharp sense for emphasising what was important in each paper, and invaluable contributions especially on the folic acid field and on statistical analyses.

Tatiana Fomina has been the amazing statistician in this project and an essential collaborator, creating (and recreating a number of times) the statistical syntax for the CHD classification, and providing various variables. I am thankful for her incredible patience! I want to thank Grethe S. Tell for important comments during the planning of each project, and thorough effort to refine the final manuscripts. I am also grateful to my other co-authors; Henrik Holmstrøm, Kristoffer Brodwall, Trude Gildestad, and Roy Miodini Nilsen, for invaluable advices and contributions to the present work.

I greatly appreciate the excellent working environment provided by the Department of Global Public Health and Primary Care. I want to thank the competent and positive staff for practical help, researcher colleagues for social, practical and professional input, and not the least Tone Bjørge and the rest of the Genetic Epidemiology research group for valuable support. I also am grateful to Kari Klungsøyr for additional help understanding the variables

from the Medical Birth Registry, and to Jarle Jortveit who through his work with the data had valuable inputs refining the classification syntax.

My thanks goes to everyone else who made my study possible; the Research Council Norway, the Meltzer Research Fund, and Helse Vest for funding and additional grants; the Cardiovascular Disease in Norway (CVDNOR) project, the Norwegian Institute for Public Health, and Oslo Universitetssykehus Rikshospitalet for providing registry data; and the Department of Heart Disease for understanding and flexibility. I want to thank Ansgar Berg, Tom Omdal, Asle Hirth and Grete Slettom among other colleagues at Haukeland University Hospital for your friendship and support.

Finally, I want to express my gratitude to my faithful friends, family and in-laws for your friendship, cheers and support, and to Otto, the love of my life, for your continuous encouragement and unfailing faith in me.

Contents

Abbreviations

AGA	appropriate for gestational age
APVR	anomalous pulmonary venous return
aRR	adjusted relative risk
ASD	atrial septal defect
AVSD	atrioventricular septal defect
BERTE	Oslo University Hospital´s database for children with heart defects
CHD	congenital heart defect
CoA	coarctation of the aorta
CVDNOR	The Cardiovascular Disease in Norway project
DORV	double outlet of the right ventricle
GA	gestational age
GDM	gestational diabetes mellitus
HLHS	hypoplastic left heart syndrome
HRHS	hypoplastic right heart syndrome
IAA	interrupted aortic arch,
ICD	The International Classification of Diseases
IVF	in vitro fertilisation
LGA	large for gestational age
LVOTO	left ventricular outflow tract obstructions
MBRN	The Medical Birth Registry of Norway
NCMP	The Norwegian classification of medical procedures
NCSP	The NOMESCO Classification of Surgical Procedures
PAS	Patient Administrative System at the hospitals
PDA	patent ductus arteriosus
RVOTO	right ventricular outflow tract obstructions
RR	relative risk

SGA	small for gestational age
SIF	the Norwegian institute of Public Health
SD	standard deviations
TGA	d-transposition of the great arteries
ToF	tetralogy of Fallot
vPS	valvular pulmonary stenosis
VSD	ventricular septal defect

List of publications

1. Leirgul E, Fomina T, Brodwall K, Greve G, Holmstrøm H, Vollset S, Tell GS, Øyen N. Birth prevalence of congenital heart defects in Norway 1994-2009—A nationwide study. *Am Heart J.* 2014;168(6):956-64

2. Leirgul E, Brodwall K, Greve G, Vollset S, Holmstrøm H, Tell GS, Øyen N. Maternal diabetes and infant risk of congenital heart defects: A nation-wide study from Norway, 1994-2009 *Submitted 2016*

3. Leirgul E, Gildestad T, Nilsen RM, Fomina T, Brodwall K, Greve G, Vollset SE, Holmstrøm H, Tell GS, Øyen N. Periconceptional Folic Acid Supplementation and Infant Risk of Congenital Heart Defects in Norway 1999-2009. *Paediatr Perinat Epidemiol* 2015;29(5):391-400.

Abstract

Background: Congenital heart defects (CHDs) are the most common birth defects, affecting 5-10 per 1000 live births. The aetiology is considered multifactorial, with both genetic and environmental causes, which are still incompletely understood. Pregestational maternal diabetes is a well-known risk factor, with 2-5-fold risk increase in offspring of diabetic women compared to children of non-diabetic mothers. The risk is believed related to glycaemic control in pregnancy. Since 1999, the birth prevalences have been reported decreasing in several European countries and Canada; the reasons for this are not known, but increased use of periconceptional folic acid supplementation and folic acid food fortification may have contributed.

Objectives: The prevalence of CHD in Norway has not previously been presented. This project aimed to present the true prevalence of CHD phenotypes among live births, stillbirths, and terminated pregnancies in the Norwegian population by year of birth, and to investigate the association of CHD risk and important modifiable risk factors: maternal diabetes mellitus and periconceptional folic acid intake.

Methods: Information on CHD diagnoses, maternal pregestational diabetes and gestational diabetes, and use of folic acid or multivitamin supplements among all births in Norway, 1994-2009 was ascertained from four data sources; The Medical Birth Registry of Norway, the Patient Administrative Systems at the hospitals, Oslo University Hospital`s clinical database for children with heart defects, and the Cause of Death Registry, as part of the multipurpose project Cardiovascular Disease in Norway (CVDNOR). Individuals with heart defects were assigned specific cardiac phenotypes. Time trend of CHD prevalence was analysed using Joinpoint Regression Program. The association between maternal diabetes and infant risk of CHD was estimated as relative risk (RR), comparing CHD risk among children of women with diabetes with CHD risk among offspring of non-diabetic women. The association between periconceptional folic acid intake and offspring CHD was analysed for births 1999-2009.

Results: Among 954,413 births (live births, stillbirths, termination of pregnancies) 1994-2009, 13,081 were diagnosed with CHD (137.1 per 10,000 births, 133.2 per 10,000 live births). Excluding patent ductus arteriosus in preterm children, the overall CHD prevalence was 123.4 per 10,000; annually, the prevalence increased with 3.5% (95% confidence interval (CI) 2.5, 4.4) in 1994-2005, and declined with 9.8% (95% CI -16.7, -2.4) from 2005 through 2009. Severe CHD prevalence was 30.7 per 10,000; per year increase was 2.3% (95% CI 1.1, 3.5) in 1994-2004, and per year decrease 3.4% (95% CI -6.6, -0.0) in 2004-2009.

Among 914,427 singleton births without chromosomal aberrations or genetic disorders, the prevalence of CHD was 116 per 10,000 births. Maternal pregestational diabetes (type 1, type 2, unspecified diabetes) had been diagnosed in 5,618 (0.61%) births, and gestational diabetes in 9,726 (1.06%) births. In offspring of women with pregestational diabetes, RRs for any CHD and severe CHD were 2.92 (95% CI 2.54, 3.36) and 3.34 (95% CI 2.48, 4.49) respectively, as compared to CHD risk in offspring of non-diabetic mothers. Among pregnancies with gestational diabetes the RRs were 1.47 (95% CI 1.26, 1.71) and 1.40 (95% CI 0.98, 1.98) for any CHD and severe CHD. The CHD risk in children of diabetic mothers did not change significantly during the study period.

Among 514,514 singleton births without chromosomal aberrations or genetic disorders 1999-2009, 6,108 were identified with CHD. Folic acid supplements before and/or during pregnancy were used in 51.2% of pregnancies. The adjusted RR of severe CHD was 0.99 (95% CI 0.86, 1.13) comparing births exposed to periconceptional intake of folic acid with no intake, while RR of septal defects was 1.22 (95% CI 1.12, 1.32).

Conclusions: The birth prevalence of CHD declined in Norway from around 2005. Specifically, the prevalence of severe CHD was reduced by 3.4% per year from 2004 through 2009. A threefold risk of CHD in children of mothers with pregestational diabetes, and a 40% risk increase in offspring of women with gestational diabetes, did not change from 1994 to 2009. Periconceptional folic acid supplement use showed no association with severe CHD in the newborn. Our finding of an unexpected association of folic acid use with an increased risk of septal defects warrants further investigation.

1. Introduction

1.1 Congenital heart defects

1.1.1 Definition

Congenital heart defects (CHD) are structural malformations of the heart or the large thoracic vessels that are present at birth. These cardiac anomalies vary from minor lesions of substantially no clinical significance, to severe conditions requiring extensive medical care, and with impaired physical ability and life expectancy. Varying definitions of CHD have been used in previous prevalence studies. In 1971, Michell et al. defined CHD as ”a gross structural abnormality of the heart or intrathoracic great vessels that is actually or potentially of functional significance”(1). In recent studies, CHDs have been defined by morphologic or anatomic diagnoses irrespective of their functional impact. However, some conditions represent remnants of foetal circulation and are therefore considered normal the first days after birth. They have been included to varying degree in previous studies, which must be taken into account when comparing prevalence numbers. The cardiac malformations can be grouped and classified by various methods, depending on the point of interest. A classification of congenital heart defects based on the embryological development facilitates epidemiological studies of the association between risk factors in pregnancy and CHD in the offspring.

1.1.2 Embryology - the developing heart

The embryo heart becomes evident in the second week after conception (often referred to as the fourth week of pregnancy). Cardiogenic mesodermal cells migrate cranially and laterally to form two endocardial tubes on each side of the neural plate (Figure 1)(2-4). As the neural plate

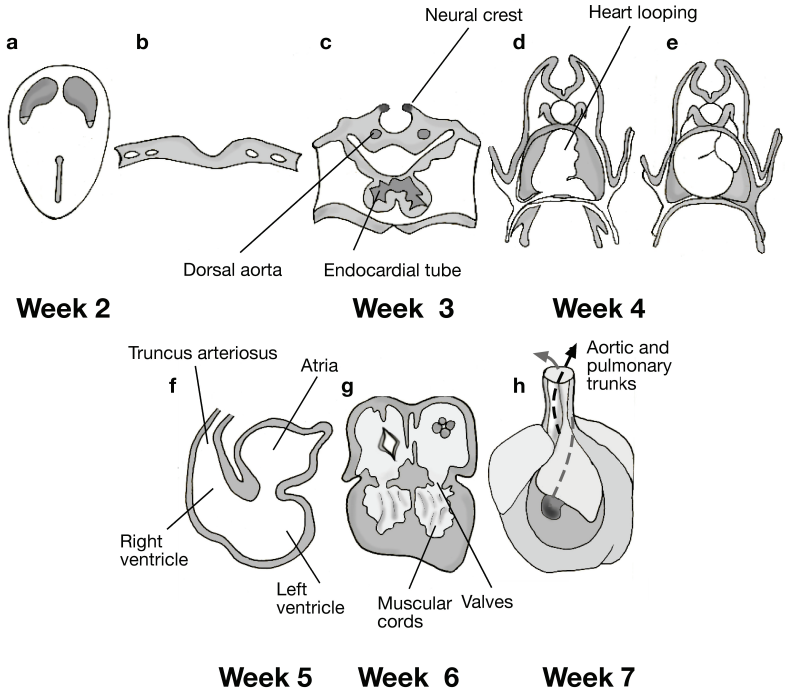
is folded dorsally to form the neural tube, the endocardial tubes converge anteriorly and fuse in the midline into a single tube. The linear heart tube begins beating around day 21. During the third week of embryonic development the tube expands anteriorly, and starts bending towards the right side to form a looping structure. In the fourth week the primitive heart tube consists of an inlet (atrium), a central chamber (primitive ventricle), and an outlet (bulbus cordis and outlet artery). Between week 5 and 8, the heart continues its clockwise rotation, septa grow into the primitive atrium and ventricle separating the tube into four chambers, and in the outflow tract separating the aorta from the pulmonary artery. However, an opening between the two atria, foramen ovale, remains until birth, allowing oxygenated blood from placenta to enter the left heart and the systemic circulation to the brain and coronary arteries(5). The heart is unique and vital, since from its early stages, it is operational with circulation established as it transforms from a single tube to a four-chambered valved organ. This development, however, is a complex process with many opportunities for error, as evidenced by the large variety of cardiac malformations.

Regions of the developing heart have been defined, which gives a foundation for better understanding of the connection between different cardiac anomalies and possible common aetiological factors(6). Cells from the first primitive midline streak migrating anteriorly and laterally, to eventually form the left ventricle of the heart constitute the primary heart field. Cells from the dorsal mesocardium give rise to the secondary heart field. During the looping process of the heart tube in the 4^{th} week of the foetal development these cells form the atria, right ventricle, and the outflow tract(7). The anterior region of the secondary heart field contributes to the right ventricle and the outflow tract, while the posterior region forms the atrial and atrioventricular septal structures. Impaired development of the secondary heart field structures may result in a number of common CHDs including conotruncal defects, ASD, and AVSD.

A group of multipotent embryonic cells, the neural crest cells, arise in the neuroectoderm on the dorsal side of the neural tube in the embryo. They migrate throughout the embryo and give rise to most of the peripheral nervous system, in addition to a number of

other cell types. The cardiac neural crest cells migrate to the 3rd, 4th and 6th pharyngeal arches and the cardiac outflow tract. Some cells continue to migrate to later form the aorticopulmonary septum(8, 9). The neural crest cells are hypothesised to be especially vulnerable for a number of teratogenic exposures (e.g. environmental toxins(10) or medications(11)), which may cause impaired outflow septation and also impact heart looping and valve modelling.

Figure 1. Development of the heart



©Gørill Skaale Johansen / Elisabeth Leirgul

*Figure 1. **a**. Precardiac cells in primitive streak the second week after conception. **b.** Folding of the neural plate. Cardiogenic areas form laterally. **c.** Folding brings heart tubes into the ventral midline. The heart tube begins beating day 21. **d and e.** Looping of the heart tube. **f.** Fusion of endocardial cushions. **g.** Development of ventricular and atrial septae. **h.** Septation into aortic and pulmonary trunks.*

1.1.3 Classification of congenital heart defects

Classification of heart defects is challenging because of the numerous different malformations, which may appear alone or in combination in a single individual, with a wide range of severity and with different underlying developmental aetiology. A number of classification systems have been developed to meet various clinical, academic, and administrative requirements. *The International Classification of Diseases (ICD) system* is maintained by the World Health Organisation and is used in administrative systems in more than 100 countries worldwide, as the international standard system for reporting of mortality and morbidity. It was created as the "International List of Causes of Death" in 1893, and has been further developed in so far 10 editions. The ICD system classifies each heart defect, as well as some established combination of defects (for instance, Tetralogy of Fallot, congenitally corrected transposition of the great arteries), based on both anatomical and functional considerations. Therefore, if surveillance registries based on this system receives records of the number of diagnoses, individuals with multiple cardiac defects will be counted several times.

In Norway the ICD system has been used in administrative systems since 1951. Heart defects reported from electronic Patient Administrative Systems (PAS) in somatic hospitals, the Medical Birth Registry (MBRN), and the Cause of Death Registry are classified according to the ICD system.

In 1984, Lodewyk H. S. Van Mierop published a descriptive diagnostic coding system based on an anatomical, segmental approach(12, 13), avoiding terms implying assumed embryological aetiology (e.g. ostium secundum type ASD), eponymic terms, and complexes (e.g. Tetralogy of Fallot, Eisenmenger complex). *The Van Mierop classification system* is an extensive system where individuals with complex heart defects are given a number of codes to describe the anatomical elements of the malformation, and also elements of normal cardiac anatomy. In addition, the coding system includes some syndromes and conditions known to be associated with CHD. A modified version of this system is used in Oslo University Hospital's clinical database for children with heart defects (BERTE)(14), alongside the ICD system.

For epidemiological research, however, it is more appropriate to assign each individual (including those with multiple cardiac defects) to specific cardiac phenotypes. Lorenzo Botto et al published in 2007 a classification system developed for etiological research, based on developmental and epidemiological considerations(15). The original classification system by Botto and colleagues was designed for clinical review of each individual. The diagnostic coding is organised in three levels. Level 1 contains detailed diagnostic codes on specific heart defects (e.g. Tetralogy of Fallot (ToF) with absent pulmonary valve, ToF with pulmonary atresia (PA), ToF with double outlet right ventricle (DORV), or classic ToF). The cardiac phenotypes are defined by Level 2 codes (e.g. ToF), and Level 3, aggregation of Level 2 groups in 8 main groups comprising defects considered etiologically related (e.g. conotruncal defects) (Appendix 1).

1.1.4 Birth prevalence of congenital heart defects

There are several measures of disease frequencies. In epidemiology, the occurrence of a disease or health issue in a population is usually estimated in terms of incidence or prevalence of the disease. *Prevalence* is the proportion of individuals in a defined population found to have the condition of interest at a specific point in time ("point prevalence") or during a given period of time ("period prevalence"). *Incidence* is the measure of the new cases of a disease or health related condition during a certain period of time, and *incidence rate* refers to the proportion of individuals acquiring the condition in the time period. The occurrence of congenital birth defects, however, is better quoted as *birth prevalence* rather than incidence(16). The exact timing of the malformation is difficult to determine, but it is in many instances thought to occur during the first few months of pregnancy. A malformation of the embryo or foetus may lead to miscarriage, and the proportion of foetuses affected by a congenital defect, e.g. the incidence of the specific birth defect, is therefore difficult to estimate. The term birth prevalence refers to the number of a specific condition in live births and stillbirths (and in some studies terminated pregnancies are included); divided by the total number of births.

Congenital heart defects are the most common birth malformations, reported to affect 5-10 per 1000 live births(17-20). There is substantial variation in the reported CHD birth prevalence by year of birth, and in different populations(20), which to a large extent is explained by the varying inclusion of trivial defects, as smaller septal defects (17). While there is little evidence for significant geographical differences in the birth prevalence of heart defects, several studies indicate a changing prevalence over time(19, 20). During the nineteen-eighties and -nineties, the recorded birth prevalence of CHD increased substantially. Improved diagnostic tools, such as high-quality ultrasound technology, may have led to increased detection of minor anomalies in this time period. Although the septal defects accounted for the largest proportion of the overall increase in CHD prevalence(19, 21), several studies also reported an increased prevalence of severe heart defects(19, 22). However, recent studies have reported that the CHD trend is changing. In Quebec, Canada(22), the prevalence of severe heart defects started to decrease from 1999. The authors suggested a preventive effect of mandatory folic acid fortification of cereal products introduced in Canada in 1998. Increasing availability of prenatal diagnostics and termination of pregnancies with a foetus affected with severe CHD could also have contributed to the drop in live birth prevalence of severe heart defects. To our knowledge birth prevalences of CHD including terminated pregnancies have not been previously reported.

1.1.5 Aetiology and risk factors for congenital heart defects

The causes of CHD are incompletely understood, and the aetiology has been considered multifactorial, with both genetic and environmental factors(23, 24). The knowledge of the genetic factors influencing the development of CHD has improved over the last decades. While chromosomal abnormalities (e.g. Down syndrome, Turner syndrome, DiGeorge syndrome) account for 7-10% of CHD(19, 25), an increasing number of single gene defects have also been associated with CHD. In addition to single gene mutations leading to syndromes with high proportion of CHD (e.g. Holt-Oram syndrome, Noonan syndrome, CHARGE syndrome), a

number of single gene defects associated with isolated CHD have been identified(26, 27). There are still unknown hereditary factors predisposing to CHD. Children with a first-grade relative with CHD have increased risk of CHD, ranging from 3-fold to 80-fold for different cardiac phenotypes in a recent Danish study(28). However, only 4.2% of non-chromosomal CHD were attributed to family history. Therefore, environmental factors and other maternal risk factors may be important in the aetiology of CHD.

Non-modifiable risk factors include maternal age(29), in vitro fertilisation, and multiple pregnancies(30). A number of modifiable risk factors have also been identified, and include maternal diseases e.g. diabetes; phenylketonuria(31); rubella(24) and other infections in pregnancy; exposure to teratogenic drugs, for example thalidomide, retinoids, and indomethacin; exposure to environmental factors like radiation, air pollution(32), or cigarette smoking(33); and nutritional factors, e.g. folate deficiency. Obesity without established diabetes mellitus has also been associated with an increased risk for CHD in the offspring(34).

In the present project, we studied the impact of two important modifiable risk factors on the prevalence of CHD in Norway; maternal diabetes mellitus, in which optimised treatment is believed to reduce the risk for CHD, and folate insufficiency in pregnancy, which is targeted by folic acid supplementation.

1.2 Diabetes Mellitus in pregnancy and offspring CHD risk

1.2.1 Pregestational diabetes mellitus

Diabetes mellitus has until recently been classified into two main types: Type 1 diabetes is defined by absolute insulin deficiency caused by destruction of pancreatic beta cells. The most common form of diabetes, type 2, is characterised by insulin resistance and a relative insulin deficiency with or without reduced insulin secretion(35). Diabetes mellitus is diagnosed by measuring random plasma glucose ≥11.1 mmol/l (200 mg/dl), fasting plasma glucose ≥7.0

mmol/l (126 mg/dl), or plasma glucose ≥11.1 mmol/l (200 mg/dl) two hours after oral intake of 75 g glucose (glucose load test)(35, 36).

Among pregnant women in Norway in 2010, the prevalences of diabetes type 1 and type 2 were 0.47% and 0.27%, respectively(37). As in most of the western world, the prevalence of Diabetes type 2 has increased in Norway over the last decades, particularly in the younger age groups(38). Optimised treatment of diabetes during pregnancy is considered essential to avoid maternal and foetal complications(39). International guidelines, therefore, recommend preconception counselling and optimised perinatal glycaemic control in women with diabetes(40). Reports from Europe indicate improving perinatal glycaemic control in women with pregestational diabetes from the nineties(41). Whether improved perinatal care of diabetic women in Norway has influenced the risk of CHD in their children is, however, not known.

1.2.2 Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is glucose intolerance presenting during pregnancy(42, 43), defined as fasting plasma glucose ≥7.0 mmol/l, or plasma glucose ≥7.8 mmol/l 2 hours after a 75 g glucose load. This definition includes impaired glucose tolerance occurring during pregnancy, and pre-existing undiagnosed diabetes. Insulin resistance normally increases during pregnancy from the second trimester. The underlying mechanisms are not well known, however, placental hormones are thought to play a major role(43, 44). GDM usually disappears after pregnancy, but in some cases persists as diabetes mellitus type 2.

Risk factors for GDM are obesity, GDM in a previous pregnancy, previous children with high birth weight (>4500 g), age, and first-grade relatives with diabetes. The prevalence of GDM is considered between 1 and 14% of all pregnancies(45, 46); the wide variety caused by differences in diagnostic criteria, screening routines, and differences in genetics and life style in various populations. The increasing prevalence of obesity in many countries seems to be followed by increasing incidence of GDM(46). Among 230,000 women giving birth in Norway

2004-2008, 1.0% were diagnosed with GDM(47). Norwegian guidelines for maternity care(48) recommend in total 8 follow-ups during pregnancy, the first in week 8-12, with testing for glycosuria. An oral glucose load test is recommended in women with glycosuria, and in week 26-28 in women with increased risk (age >38 years, with first-grade relatives with diabetes, pre-pregnancy BMI>27 kg/m2), previous GDM, and immigrants from countries with high prevalence of diabetes).

1.2.3 Maternal diabetes and congenital heart defects

Pregestational diabetes mellitus is a well-established risk factor for a number of congenital malformations(49), with a 2-5-fold increased risk for CHD in the offspring(50, 51). The risk has been shown to correlate with glucose levels during pregnancy, with high risk for CHD in births after pregnancies with poor glycaemic control measured by glycated hemoglobin (HbA1c) in type 1 diabetes(52) or by fasting glucose level in women with type 2 diabetes or GDM(53).

During a normal pregnancy, increased secretion of cortisol, in addition to several pregnancy-related hormones, causes increased insulin resistance. Therefore, to maintain normal blood glucose values, the insulin secretion is doubled throughout a normal pregnancy(54). While maternal insulin does not cross the placenta, glucose is transported across the placenta by facilitated diffusion via hexose transporting GLUT proteins(55). This diffusion seems to be limited when maternal glucose level exceeds 12-13 mmol/l(56).

The teratogenic effects of diabetes are not clear. Although foetal hyperglycaemia is suggested to be the major teratogen in diabetic pregnancies, other metabolic factors may contribute to the embryopathy; e.g. high levels of ketone bodies and triglycerides, inhibition of foetal myoinositol uptake, or free oxygen radicals(57, 58). High foetal glucose levels are hypothesised to change the expression of a regulatory gene in the embryo leading to cell death of neural crest cells and abnormal neural crest cell migration(49, 59), and impaired development of cells in the secondary heart field. Whether high levels of foetal insulin and other growth-stimulating factors plays a role in the aetiology of CHD is not known.

1.2.4 Maternal diabetes affects foetal growth

Macrosomia can be defined as birth weight >4000 g or > 4500 g, >90 percentile, or >+2SD(60). Macrosomia has been reported as a complication in as much as 35%-70% of infants in pregnancies with maternal pregestational diabetes(54), and pregnancies with gestational diabetes in around 30%(61, 62). The foetal pancreas develops from the end of the first trimester, producing measurable insulin levels around week 19(63). Hyperglycaemia induces overstimulation of the foetal pancreatic b-cells and high levels of insulin and insulin-like growth factors, which stimulates growth of foetal fat tissue, muscle cells, and viscera. A study of women with GDM treated with insulin and a tight control of blood glucose showed no increased risk of macrosomia compared with non-diabetic pregnancies(64); in addition, obese women with this treatment showed lower risk of macrosomia than obese non-diabetic women. Maternal obesity is a risk factor for type 2 diabetes and gestational diabetes, but also non-diabetic overweight mothers have an increased risk for infants with high birth weight(65). Voldner et al showed a 4.5-fold increase in risk of infant macrosomia in overweight women without diabetes, but with increasing fasting glucose during pregnancy, compared to the remaining group of overweight women(66). Maternal insulin resistance might therefore be an important cause of the association between obesity and foetal macrosomia.

As foetal macrosomia could be an indicator of hyperglycaemia, analyses of the association between high birth weight and CHD in children of diabetic women could be used to investigate the hypothesis that hyperglycaemia is the main cause of the increased risk for offspring CHD in diabetes pregnancies. The association between macrosomia and CHD in children of diabetic mothers is to our knowledge not previously presented.

Contrary growth inhibition has been observed due to maternal diabetes complicated with vasculopathy and nephropathy(67), or severe hyperglycaemia with B-cell exhaustion and foetal hypoinsulinemia(68).

1.3 Folic acid and folate

1.3.1 Definition

Folate is a water-soluble B vitamin (vitamin B_9) that is necessary for the synthesis of purine and thymidine nucleotides, and for the synthesis of methionine from homocysteine(69). It occurs in structurally related and interconvertible enzyme cofactors, distinguished by their oxidation state and the type of one-carbon substitution. The fully oxidised and chemically stable synthetic form of folate referred to as folic acid, is used in dietary supplements, but does not occur naturally to any significant extent(69, 70). Folate is naturally present in fruits and vegetables, especially dark green leafy vegetables (e.g. spinach, broccoli, cabbage) and citrus fruits, in beans and lentils, yeast, liver, whole grain flour, and dairy products.

1.3.2 Folate in foetal development

Folate is essential for the synthesis of nucleic acids, and also for methylation of DNA, RNA, proteins and phospholipids, and critically important for DNA synthesis, cell replication and growth. It is therefore particularly important during pregnancy, where folate requirements are increased 5- to 10-fold compared to non-pregnant women(71).

In mouse models, neural crest cells have been shown to be rich in high-affinity folate receptors, and transient suppression of these receptors during a critical stage of the neural crest development to severely affect the cardiac development(72). Folate deficiency during the first part of the pregnancy might therefore inhibit neural crest cell migration, and increase the risk for conotruncal defects.

Folate is actively transferred from maternal to foetal circulation by folate receptors and folate binding protein carriers in placenta, and the intervillous folate concentration is found to be three times that of the maternal blood(73).

1.3.3 Folate and risk of neural tube defects

The brain and spinal cord develop from a neural tube formed by infolding of ectodermal tissue around two weeks after conception. The doubling time of the embryonic neural cells is only 5 hours, and folate deficiency during the critical time window is therefore likely to have a major impact on the neural tube development(71). Several studies have shown significant risk reduction of neural tube defects in offspring of mothers using folic acid supplements from before conception throughout the first trimester of pregnancy(74-77). In a community-based study from China 1993-1995, the risk for having a child with neural tube defect was reduced by 80% in the high-prevalent northern region, and 40% in the low-prevalent southern region of the country, in women taking 0.4 mg folic acid before conception(78).

1.3.4 Folate and risk of congenital heart defects

A protective effect of periconceptional folic acid supplementation on risk of CHD has also been suggested. While neural tube defects are severe, but rare birth defects, CHD affects more than 1% of all newborn babies. Therefore, a possible risk reduction by folic acid supplementation or food fortification would have a major health impact in society. Studies on the association between folic acid/ multivitamin use before pregnancy and other births defects have been undertaken, with particular attention on defects originating in tissue under neural crest cells control, such as the conotruncal area of the heart. The results are, however, still inconclusive(79).

Only a few studies have reported individual level information of maternal folic acid supplement use and infant risk of CHD. A Californian case-control study from 1995(80), based on telephone interviews with the mothers of 207 children with conotruncal heart defects and of 481 randomly selected infants without malformations, reported reduced risk for conotruncal heart defects in children of mothers who had taken multivitamins or folic acid fortified cereals. In a Hungarian randomised placebo controlled trial with 5574 pregnant women(81), multivitamin supplements with 0.8 mg folic acid were compared to supplements

with other trace elements. There was significantly reduced risk for CHD, especially conotruncal defects, in the group receiving vitamins with high-dose folic acid, but the numbers were small, with only 10 vs.20 cases of any type of CHD in the exposed and unexposed group, respectively. A registry-based case-control study from the Northern Netherlands 1996-2005(82), including 611 children with CHD, and two control groups; 2401 children with supposedly non-folate related birth defects, and 3343 children of women participating in previous cross-sectional studies, reported a reduced risk for CHD, mainly septal defects, in offspring of women using periconceptional folic acid supplements (0.4 mg/d).

Canada implemented mandatory folic acid fortification of cereal products from 1998. A time trend analysis of severe CHD in children born in Quebec, Canada, 1990-2005 showed decreasing prevalence of severe heart defects from 1999(22). However, comparing congenital malformations in pre-fortification period (1992-1996) to post-fortification period (1999-2003) in Alberta, Canada(83), decreasing prevalence for ostium secundum ASD was found, but no change in prevalences of VSD, ToF, transposition of the great arteries (TGA), truncus arteriosus or CHD combined. Correlation between the time trends of folic acid supplement use or food fortification, and the birth prevalence of CHD in populations should be interpreted with caution, since the two events are not necessarily causally related. To our knowledge, there are no previously published population-based cohort studies with individual information on periconceptional supplement use.

1.3.5 Folic acid supplements in pregnancy

Since the 1990s, women worldwide have been recommended to take folic acid supplements of at least 0.4 mg daily when planning a pregnancy and during the first trimester to reduce the risk of neural tube defect in the child(84).

1.3.6 Folic acid fortification

Food fortification refers to the practice of adding essential vitamins and minerals to staple foods such as flour, salt, sugar, and rice to prevent or correct a nutrient deficiency in the population. Folic acid supplements are likely to have a preventive effect on neural tube defects only when taken during the first 2-3 weeks of pregnancy. Because many women will initiate supplementation too late to achieve risk reduction(85) more than 70 countries (for example, Australia, Canada, USA, Indonesia) have implemented mandatory fortification of grain and cereal with folic acid(22), yet still not any European country. As risk reduction was suggested for congenital heart defects, which are the most common birth defects, the question of folic acid food fortification has become actualised in Europe(86).

1.3.7 Folic acid supplementation in Norway

While there has been no mandatory food fortification of folic acid in Norway, a few food production companies have obtained permission to enrich specific products with folic acid(87). Since 1998, Norwegian health authorities have recommended periconceptional folic acid supplements with intake of 0.4 mg folic acid once daily when planning a pregnancy and during the first trimester(88). Intake of folic acid and multivitamin supplementation has been recorded in MBRN since 1999(89).

Folic acid supplements containing 0.4 mg folic acid are for sale over the counter in Norwegian pharmacies, health food stores, and many grocery stores. Supplements containing 1 mg folic acid require a medical prescription. Women with neural tube defects, or who had previous children with neural tube defects, and women using folate-reducing medication (i.e. anti-epileptic drugs) are recommended periconceptional intake of 4 mg folic acid supplements.

2. Objectives of the thesis

The present thesis aimed to study prevalences and time trends of congenital heart defects in Norway and investigate the effect of selected risk factors in the Norwegian population.

The main objectives were:

To develop methods for ascertaining information on individuals with congenital heart defects (CHD) in Norway, and for classifying these individuals with CHD into cardiac phenotypes, and to describe the birth prevalence of CHD in Norway.

To investigate the associations of pre-gestational diabetes or gestational diabetes and offspring risk of CHD, to evaluate whether non-optimal glucose regulation, measured as foetal growth, modify the risk of CHD in diabetic and non-diabetic pregnancies, and to investigate time trends of CHD risk in diabetic pregnancies as compared to non-diabetic pregnancies.

To investigate the association of mother's self-reported intake of folic acid and multivitamin supplements before and/or during pregnancy and offspring risk of CHD.

3. Material and methods

3.1 Data sources

3.1.1 The National Registry

The Norwegian National Registry has since 1965 registered demographic data on all residents in Norway with information on name, address, marital status, births, and deaths. Every person is registered with a unique personal identification number, which enables linkage of information between administrative data sources and national health registries and clinical registries.

3.1.2 The Medical Birth Registry of Norway

The Medical Birth Registry of Norway (MBRN) was established after the "Thalidomide disaster"(90) in 1967 for the purpose of monitoring the prevalence of birth defects and providing a basis for epidemiological research (www.fhi.no). MBRN is a national health registry with compulsory notification of all births including medical information of the mothers' health before and during pregnancy, the course of delivery, and the health of the newborn child. Until 1998, live births and stillbirths from 16th week gestation were reported to the registry. Only few terminated pregnancies were reported, and may in some cases have been registered as stillbirths(91). Since 1999, reporting of all pregnancies terminated because of foetal anomalies after gestational week 12 has been mandatory, and from 2002 all births from 12th week gestation(92, 93). The information is collected from an antenatal chart and from hospital records at the time of delivery, and registered in a standardised notification form by midwives and physicians attending the mother and child at delivery and the following days until discharge (Appendix 2). A revised version of the notification form was introduced in December 1998 collecting new information including maternal smoking and folic acid or multivitamin

supplement intake (Appendix 3). In addition to pre-coded fields with check boxes, the notification includes ICD diagnoses (ICD-8 from 1994 through 1997, and ICD-10 from 1997 onwards) and text fields. Since 1999, MBRN has been updated with information on diagnoses and causes of death to the age of one year.

3.1.3 The Patient Administrative System at the hospitals (PAS)

The multipurpose research project Cardiovascular Disease in Norway (CVDNOR)(94) was established in collaboration between the University of Bergen and the Norwegian Knowledge Centre for the Health Services. Information on cardiovascular diseases including congenital heart defects and diabetes mellitus was retrieved from the electronic Patient Administrative Systems (PAS) of all somatic hospitals in Norway, 1994-2009(95). Every hospital stay with any discharge diagnosis or surgical procedural code related to the cardiovascular system or diabetes (up to 20 discharge diagnoses for each hospital stay) with the corresponding date of discharge was included in the database. Diagnoses from outpatient clinics were not included. PAS records diagnoses by the ICD system, 9th Revision (ICD-9) from 1994 through 1998, and ICD-10 from 1999 onwards, and interventions by the NOMESCO Classification of Surgical Procedures (NCSP) the Norwegian classification of medical procedures (NCMP).

3.1.4 Oslo University Hospital´s clinical database for children with heart defects (BERTE)

Oslo University Hospital's clinical database for children with heart defects is called BERTE, which is short for "Barne-hjerte", i.e. child heart(14).

BERTE contains information on all children with a heart condition who have been examined by a paediatric cardiologist or have received surgery or intervention at Oslo University Hospital since 1992(14). The date of diagnosis is not registered in BERTE, and diagnoses may be revised at follow-up consults. BERTE reports diagnoses by the Van Mierop classification system and the ICD system, in addition to text fields. Procedures and

interventions are dated and coded by the NOMESCO Classification of Surgical Procedures (NCSP) and by codes from the Norwegian institute of Public Health (SIF).

BERTE was originally constructed in 1989 as an electronic system of medical notes containing the most important information concerning the cardiac condition and supplementing the paper medical note system used at the time. In 2002, BERTE underwent a major upgrade to a windows-based system. The software was developed by Victoria Data, a firm now known as DIPS. DIPS is now the leading supplier of electronic patient journal systems in Norway. In addition to codes for diagnoses and medical or surgical procedures the database contains text fields. The short text entered on each patient for every contact was restricted to 60 characters intended to give an instant summary of the medical history. It is characterised by extensive use of abbreviations and acronyms, and the information is not easily accessible. Information on extracardiac conditions is to a large extent only available in the text fields and with numerous different terms. For example, Down syndrome is noted by “down”, “ds”, “trisomy 21”, “trisomi21”, and “trisom 21” with a variety of uppercase and lowercase letters, while variants of the terms “non-ds” and “non-down” must be excluded when searching for string variables indicating this condition.

3.1.5 The Cause of Death Registry

Causes of death have been systematically registered and analysed in Norway since 1853, from 2001 by the Norwegian Institute of Public Health(96). The Cause of Death Registry contains information about the underlying cause of death and up to six contributing causes of death, as recorded from the death certificate by the ICD system (ICD-9 from 1994 through 1995, and ICD-10 from 1996 onwards). This registry provided information about deaths not registered in MBRN.

3.1.6 Statistics Norway

Statistics Norway has the overall responsibility for official statistics regarding the Norwegian society and provides demographic data for all residents, including variables relevant for the present project; educational level, occupation, income, and marital status(97).

3.2 CHD ascertainment and classification

3.2.1 Case ascertainment

An individual was considered to have a CHD if registered with any ICD or Van Mierop code corresponding to a CHD diagnosis or medical and surgical procedures indicating a specific CHD (Table 1).

Codes for surgical or medical procedures that could have been performed for conditions other than congenital heart defects were not included. Some conditions regarded as normal in the neonatal period, but nevertheless might have been diagnosed, were persistent ductus arteriosus (PDA), and persistent foramen ovale (PFO), which may be diagnosed as atrial septal defect (ASD) in ICD. Transient peripheral pulmonary stenosis is common in healthy infants(98) and usually considered physiological, but may be diagnosed as pulmonary stenosis (PS). These diagnoses were, consequently, included only if recorded after 6 weeks of age, or if they were treated by surgery or catheter based interventions.

Table 1. CHD ascertainment by diagnosis and procedure codes		
Diagnosis codes	ICD-8	74600 – 74799, 75900 – 75901, 75909
	ICD-9	745.00 - 747.99, 759.3
	ICD-10	Q20.0 - Q28.9, Q89.3
	Van Mierop	010.0 - 012.0, 020.0, 021.0, 100.2, 744.2
Procedure codes	NCSP	FDA00, FDA10, FDA96, FDB03, FDC00, FDC10, FDC20, FDC96 FDD00, FDD10, FDD13, FDD20, FDE00, FDE10, FDE20, FDE31, FDE32, FDE96 FDG00, FDG10, FDH00, FDH10, FDH30, FDH40 FDJ00, FDJ10, FDJ20, FDJ30, FDJ42, FDJ96 FFC00, FFC10, FFC22, FFC32, FFC50, FFC60, FFC96, FFC96 FFD00, FFD20, FFD96, FFF00, FFF10, FFF20, FFF96 FFG00, FFG10, FFG20, FFG30, FFG96, FFJ00, FFJ10, FFJ96, FGB00, FGB10, FGB96 FHB00, FHB10, FHB20, FHB30, FHB40, FHB42, FHB50, FHB60, FHB70, FHB80, FHB96, FHC00, FHC10, FHC20, FHC30, FHC96 FHD00, FHD03, FHD10, FHD30, FHD96, FHE00, FHE10, FHE20, FHE30, FHE40, FHE96, FHF00, FHF10, FHF20, FHF30, FHF96, FHG00, FHG10 FHH00, FHH00, FJE00, FJE10, FJE20, FJE30, FJE42, FJE96 FMA00, FMA10, FMA20, FMA32, FMA96, FMB10, FSD96
	SIF codes	3005.0, 3011.0 - 3011.2, 3012.0 - 3012.2, 3013, 3014.1, 3014.2 3015.1 - 3015.5, 3016.0, 3029, 3031.0, 3032.0, 3039 3040.0 - 3040.4, 3041.0 - 3041.4, 3044.1, 3044.2, 3047.0, 3048.0, 3055.0 3056.0, 3056.1, 3057.0, 3057.1, 3069, 3071, 3072, 3072.2 3081.1, 3082.1, 3082.3, 3097.0, 3098.0, 3099.0, 3132.0, 3135.0, 3136.0 3136.3, 3145.0, 3145.2, 3147.0 - 3147.4, 3148.0 - 3148.3, 3149.0, 3154.0 3159.0, 3175.2, 3179.0, 3230.1 - 3230.4, 3246.1, 3249.0, 3250.0, 3251.0 3295.4, 9004.1, 9004.3, 9004.4, 9010.1, 9010.2
ICD The International Classification of Diseases NSCP The NOMESCO Classification of Surgical Procedures SIF The Norwegian institute of Public Health		

3.2.2 Key principles of the classification

The CHD diagnoses and cardiac procedures were registered by several coding systems in our data sources. In order to gather and compare the data from the different sources, we had to convert these codes into one, uniform system. We chose to base our classification on a system designed for etiological studies proposed by Lorenzo Botto and co-workers in 2007 (Appendix 1), categorising the specific heart defects according to the assumed embryological timing of the CHD development(15). We have revised and adapted this system according to the data available in our data sources. Several CHD diagnoses originally excluded by Botto et al were implemented to achieve full ascertainment of CHD, in order to be able to present true birth prevalences of CHD in Norway. In addition, while Botto et al developed a system intended for small studies with classification by clinical assessment of each individual, extensive work was needed to reclassify and prioritise CHD diagnoses defined by a number of classification systems and text fields in several data sources to this hierarchical classification system.

3.2.3 Revision of Botto´s classification system

Because the coding of diagnoses in this project was not based on an individual review, as intended by Botto and colleagues, but on diagnosis codes from several registries, a number of less precise diagnoses were collected (e.g. malformations of the great arteries or veins). These were put in the Level 3 category “Non-contributable”. A number of specific diagnoses excluded by Botto and colleagues were also implemented in the classification system. Double outlet left ventricle (DOLV) is a very rare heart defect with a complex and uncertain embryological origin, and was therefore not included in the original classification system by Botto et al(15). We chose to categorise this diagnosis as “other complex CHD”. Isolated congenitally corrected transposition of the great arteries was also included in this group. Cor triatriatum and coronary anomalies were classified as “otherwise specified”, aortopulmonary window as “conotruncal defect”, and mitral stenosis as “left ventricular outflow tract obstruction” (LVOTO). Isolated valve defects not classified as LVOTO or right ventricular outflow tract obstruction (RVOTO)

were included and classified in a new Level 3 category. In addition, we included a category of unspecified malformations. PDA was also not included in the original classification. We chose to classify PDA in a new Level 3 category with the lowest priority, and with subgroups for PDA in children born preterm or at term.

3.2.4 Diagnostic coding systems in our data sources

A number of different coding systems were used in our data sources. The International Classification of Diseases was the major source of diagnoses. The 10th edition (ICD-10) was used in all sources after it was introduced, with a transition time from previous versions in the years 1995-1998. The 9th edition (ICD-9) was used in PAS through 1998, and in the Cause of Death Registry through 1995, whereas MBRN used ICD-8 until the transition to ICD-10 in 1997. ICD-10 is also used in BERTE, but this register mainly relies on the diagnostic coding system that was developed by Lodewyk H.S. Van Mierop. Data on surgical procedures performed on individuals was given by the NOMESCO Classification of Surgical Procedures (NCSP) and by codes from the Norwegian institute of Public Health (SIF) in PAS, MBRN, and BERTE.

3.2.5 Translation of original codes into the classification system

We have reviewed every code relevant for CHD diagnoses in the four data sources mentioned in Chapter 3.1, and assigned these codes to corresponding code numbers in the revised classification system. This work resulted in translation keys, listing all diagnoses or combination of diagnosis codes in the ICD-10, ICD-9, ICD-8, and Van Mierop systems, as well as the procedures by NCSP and SIF codes, with corresponding codes in the classification system. The diagnoses or combinations of diagnoses were prioritised according to the classification hierarchy. From the resulting tables, it is possible to read out a level 1, level 2, and level 3 phenotype for any diagnostic or procedural code (or combination of codes) in each data source. In the present project, the level of precision was level 2 and 3 (Table 3).

Table 3. The classification system by level 2 and level 3 phenotypes	
Level 3	**Level 2**
Heterotaxy	Situs inversus Isomerism Dextrocardia or levocardia with other CHD
Conotruncal defects	Truncus arteriosus Transposition of the great arteries (TGA) Tetralogy of Fallot (ToF) Pulmonary atresia with ventricular septal defect (ToF type) Double outlet right ventricle (DORV) Conotruncal VSD Aortopulmonary window
Atrioventricular septal defects (AVSD)	Atrioventricular septal defects
Anomalous pulmonary venous return (APVR)	Total anomalous pulmonary venous return Partial anomalous pulmonary venous return
Left Ventricular Outflow Tract Obstructions (LVOTO)	Hypoplastic left heart syndrome (HLHS) Mitral valve stenosis Coarctation of the aorta (CoA) Interrupted aortic arch type A Valvular aortic stenosis
Right Ventricular Outflow Tract Obstructions (RVOTO)	Tricuspid atresia / stenosis Hypoplastic right heart syndrome (HRHS) Ebstein anomaly Valvular pulmonary atresia (not ToF anatomy) Arterial pulmonary atresia Valvular pulmonary stenosis
Septal defects	Atrial septal defects (ASD) Ventricular septal defects (VSD) ASD + VSD Otherwise specified or not specified septal defects
Other complex cardiac defects	Congenitally corrected transposition of the great arteries (ccTGA) Single ventricle (non-HLHS, non-HRHS) Double inlet left ventricle (DOLV) Absent PV
Isolated valve defects (not classified as LVOTO/RVOTO)	Infundibular pulmonary stenosis Pulmonary insufficiency Subaortic stenosis Aortic insufficiency Mitral insufficiency Unspecified valve malformations
Non-contributable- specified	Pulmonary arterial stenosis Other malformations of the aorta or pulmonary artery Other malformations of the great veins Cor triatriatum Coronary malformations Other specified malformation of the heart
Non-contributable- unspecified	Unspecified malformations of the heart Unspecified malformations of aorta Unspecified malformations of the pulmonary artery Unspecified malformations of the great veins
Isolated Patent ductus arteriosus (PDA)	Isolated patent ductus arteriosus (PDA)

3.2.6 Prioritising of sources and coding systems

The hierarchical classification system provides one single CHD phenotype for each individual, based on an isolated cardiac defect or a combination of defects as coded in the data sources. However, as the diagnoses registered in the various sources might differ, a prioritised selection was necessary. We prioritised the data on each individual according to assumed accuracy of registration and the precision of the coding system used (Table 4). The Van Mierop diagnoses from BERTE were given the highest priority, as they were entered and updated regularly by paediatric cardiologists. In addition, the Van Mierop diagnoses in BERTE allow more detailed classification of CHD than is possible with ICD. However, as the ICD-10 coding in BERTE showed some systematic errors and extensive use of nonspecific diagnoses, these were given relatively low priority. Priority was given to type of data source and coding system in the following order: BERTE (Van Mierop coding), PAS, MBRN, the Cause of Death Registry, and BERTE (ICD coding). The latter versions of ICD were given priority over the former. Information from birth clinics, paediatric departments, and termination notification forms are separately labelled in MBRN. In case of differing codes, diagnoses from paediatric departments were prioritised.

Individuals hospitalised more than once during the period 1994-2009 would have multiple entries in PAS. We decided to give priority for diagnoses from the university hospitals with facilities for paediatric cardiac surgery and invasive procedures, and to the first hospital stay at either a university hospital or a local hospital. If procedure codes indicating a specific heart defect were reported in individuals without any appropriate diagnose, these were finally extracted, and the individual classified according to the indicated condition.

The date of diagnosis was estimated by information in PAS (the date of first discharge with a CHD diagnose), BERTE (the date of first CHD-related procedure), and birth clinic MBRN forms (date of birth).

Table 4. Case ascertainment- source priority		
Priority	**Source**	**Diagnostic system**
1	BERTE	Van Mierop
2	PAS 1st entry university hospital	ICD-10
3	PAS 1st entry university hospital	ICD-9
4	PAS 1st entry any hospital	ICD-10
5	PAS 1st entry any hospital	ICD-9
6	MBRN-B (from paediatric department)	ICD-10
7	MBRN-F (from birth clinic)	ICD-10
8	MBRN-A (from termination notification)	ICD-10
9	Cause of Death Registry	ICD-10
10	BERTE	ICD-10
11	Cause of Death Registry	ICD-9
12	MBRN-F	ICD-8
13	BERTE	NCSP / SIF-codes
15	PAS	NCSP / SIF-codes
16	MBRN	NCSP
BERTE Oslo University Hospital´s clinical database for children with heart defects PAS Patient Administrative System MBRN The Medical Birth Registry of Norway ICD International Classification of Diseases SIF The Norwegian institute of Public Health NCSP The NOMESCO Classification of Surgical Procedures		

3.2.7 The final CHD phenotype

The translation keys provided by the coding tables were programmed in SAS to attribute every individual with a code number in our classification system, level 2 and level 3.

Diagnostic and procedural codes where extracted separately for all coding systems in the four sources and converted into our classification system, resulting in one level 2 and level 3 code for each coding system. The code from the highest prioritised source was then assigned to each individual.

In total 89 children who had been registered with diagnosis codes for lethal conditions and alive at age 2 years, but without registered heart surgery or invasive procedure, were reclassified to unspecified CHD, assuming their heart defect had been misclassified. In order to search for systematic errors in the algorithm, and also get an impression of the quality of the data, we performed a manual review of the classification of CHD cases. For each of the four data sources, 10% of the CHD cases were randomly selected and all the original heart defect codes registered in the data source were manually reviewed. In this procedure, only 2 obviously misclassified cases were identified.

3.2.8 Severe congenital heart defects

Severe CHD was defined as heterotaxia, conotruncal defects, atrioventricular septal defect (AVSD), anomalous pulmonary venous return (APVR), LVOTO, RVOTO (except valvular pulmonary stenosis), or other complex cardiac defects. With the exception of inclusion of heterotaxia in our definition, this group corresponds closely to the severity group II and I as defined by EUROCAT (single ventricle, hypoplastic left heart syndrome, hypoplastic right heart syndrome, Ebstein anomaly, tricuspid atresia, pulmonary valve atresia, truncus arteriosus, AVSD, aortic valve atresia/stenosis, transposition of great arteries, tetralogy of Fallot, total anomalous pulmonary venous return, coarctation of the aorta)(99).

3.2.9 Chromosomal aberrations, genetic disorders, and extracardial malformations

Information on chromosome aberrations and genetic conditions associated with CHD was retrieved from the four data sources mentioned in chapter 3.1 using ICD codes (8th revision 759.3-759.5, 9th revision 758.0-759.9, 10th revision D82.1, Q87.1, Q87.2, Q90.0-Q99.9), and Van Mierop codes (8000-8004, 8009-8025, 8072). In addition, we searched for notes indicating chromosomal disorders and relevant genetic conditions (e.g. Noonan, DiGeorge, Williams,

Holt-Oram, Down, Turner, etc.) in text entry fields in BERTE and the Medical Birth Registry of Norway.

CHD children with extracardiac malformations were identified by ICD codes (8^{th} revision 740.0-745.9, 748.0-756.9, 759.8-759.9, 9^{th} revision 740.0-744.9, 748.0-756.9, 759.0-759.9, 10^{th} revision Q00.0-Q18.9, Q30.0-Q89.9) and Van Mierop codes (8041-8053, 8066, 8074-8076, 8079, 8099), in addition to selected congenital malformations registered in MBRN. The categories chromosomal aberrations and extracardiac malformations were not mutually exclusive.

3.3 Exposure variables in the present project

3.3.1 Diabetes Mellitus

Diagnoses of maternal pregestational diabetes (type 1, type 2, unspecified) and gestational diabetes were ascertained from MBRN, and from the PAS data.
Diabetes mellitus was recorded in MBRN by check boxes on the standard notification form. In the form used until 1998, pregestational or gestational diabetes were recorded, but the revised form from 1999 provided separate boxes for pregestational diabetes type 1, pregestational diabetes type 2, and gestational diabetes. Diagnoses of diabetes were also noted by ICD-codes for maternal health. In addition, any antidiabetic medication during pregnancy was registered by a yes/no variable in MBRN. We did not have information on the type of antidiabetic medication.

Pregestational diabetes was identified by ICD-10 codes E10.0-E11.9, E13.0-E14.9, O24.0, O24.1, O24.3, and ICD-9 codes 250.0-250.9; diabetes type 1 by ICD-10 E10.0-E10.9, O24.0, and ICD-9 250.1; and diabetes type 2 by E11.0-E11.9, O24.1 and ICD-9 250.2. Gestational diabetes was identified by ICD-10 codes O24.4, O24.9, and ICD-9 code 648.8 (Table 4). Information on maternal diabetes from MBRN was supplemented with information on diabetes from the PAS data, with separate variables for diagnoses registered before estimated

time for conception (date of birth minus gestational age) or during pregnancy. In pregnancies with missing data on gestational age, we approximated gestational age for terminated pregnancies (23 weeks) and live births or stillbirths (40 weeks). Diagnoses of type 1 or type 2 diabetes were given priority over unspecified diabetes, and if registered before pregnancy also over gestational diabetes, while all diagnoses of diabetes registered for the first time during pregnancy were categorised as gestational diabetes. Women with diagnoses of both type 1 and type 2 diabetes were classified as having unspecified diabetes. As antidiabetic medication may be used as treatment for other conditions than diabetes (i.e. metformin in polycystic ovary syndrome), women recorded with use of such medication but no other indication of diabetes diagnoses were excluded from the analyses.

Table 4. Diagnoses of diabetes mellitus

	Pregestational diabetes mellitus type 1	**Pregestational diabetes mellitus type 2**	**Pregestational diabetes mellitus unspecified**	**Gestational diabetes mellitus**
ICD-9	250.1	250.2	250.0, 250.3-250.9	648.8
ICD-10	E10.0-E10.9 O24.0	E11.0-E11.9 O24.1	E13.0-E13.9 E14.0-E14.9 O24.3	O24.4, O24.9

ICD The International Classification of Diseases

3.3.2 Birth weight

Birth weight and gestational age at birth was registered in MBRN. Z-score for birth weight was estimated for children with recorded gestational age 23-44 weeks, based on gender-specific reference values from Norway(100). Children with birth weight < -1.28 (<10th percentile) were considered small for gestational age (SGA), and children with birth weight >+1.28 SD (>90th percentile) large for gestational age (LGA)(101).

3.3.3 Folic acid and Multivitamins

Information on intake of folic acid or multivitamin supplements has been recorded on MBRN´s standardised notification form for live births and stillbirths from 1999 onwards by 5 check boxes for use of folic acid before pregnancy, folic acid during pregnancy, multivitamins before pregnancy, and multivitamins during pregnancy, or no use of dietary supplement. The doses or duration of use were not registered in MBRN; however, folic acid supplements for common sale in Norway during the study period contained 0.4 mg folic acid, while multivitamin tablets contained a variety of vitamins and doses of folic acid of 0.1 - 0.4 mg(102). MBRN has not registered information on folic acid or multivitamin supplements for terminated pregnancies.

3.3.4 Other variables

Information on year of birth, maternal age, parity, marital status, multiple births, and maternal smoking was registered in MBRN. The date of birth and information of mother´s birthdate has been recorded on the birth notification form, and maternal age at time of delivery registered by 5-years interval (<20, 20-24, 25-29, 30-34, 35-39, >40 years). Previous pregnancies were recorded by the number of previous live births, stillbirths >24 weeks, miscarriage/stillbirths 12-23 weeks, and miscarriage <12 weeks, and parity registered in MBRN by 0, 1, 2, 3, 4, >4 previous pregnancies. Marital status was recorded by check boxes (married, cohabitant, unmarried/single, separated/divorced/widow, other). Maternal smoking was registered from 1999 by 7 check boxes (6 boxes for daily, occasional, or no smoking at the beginning and at the end of pregnancy respectively, and one box for no consent to register smoking information). Information on maternal education and family income was registered in the National Registry.

3.4 Study population

Paper 1. Congenital heart defects in Norway

All births in Norway registered in MBRN from 1994 through 2009, in total 954,413 births, were followed until 31st December 2009 for information on congenital heart defects registered in PAS, until 31st December 2010 for MBRN, BERTE, and the Cause of Death Registry.

Paper 2. Maternal diabetes and congenital heart defects

From the study population in paper 1 (954,413 births and 13,082 CHD), we excluded children with chromosomal disorders (n=3,103) and multiple births (n=33,380), in total 36,378 (3.8%), 325 infants of mothers using antidiabetic medication without any registered diagnosis of diabetes, and 3,283 children without information on maternal health, leaving 914,427 births as the study population in paper 2. In the birth weight analyses, we further excluded 34,862 children with missing information on birth weight and/or gestational age, 1,578 children with gestational age <20 weeks, 328 with gestational age >44 weeks, and 844 children registered with birth weight z-score >5.0, leaving 876,815 individuals and 10,575 births with CHD for the analyses.

Paper 3. Periconceptional folic acid and congenital heart defects

In the period 1999-2009, 652,977 births were registered in MBRN. We excluded births with chromosomal disorders (n=2,245), multiple births (n=23,815), births from in vitro fertilisation (n=15,791), and births with maternal epilepsy (n=4,978), in total 41,292 (6.3%) births. Among the remaining 611,685 births, we excluded 93,901 (15.4%) births without information of folic acid or multivitamin supplementation (which included the 1,442 pregnancies terminated for foetal reasons), leaving 517,784 live births and stillbirths (6,200 with CHD) for analyses.

3.5 Statistical analyses

Several statistical models are available to describe the association between exposure and outcome, measured by relative risk (RR) or odds ratio (OR). In cohort studies, RR can be calculated directly by incidence or birth prevalence (i.e. the ratio of birth prevalence in the exposed group and the birth prevalence in the unexposed group). Adjusted RR may be estimated by generalized linear regression with a log link and binomial distribution(103). The association measures are, however, conventionally often estimated by logistic regression, reporting the OR (i.e. the ratio of the odds of an outcome in the exposed group and the unexposed group). In case of rare outcome, OR approximates RR estimates. In the present project, estimates of OR and RR were similar, and either method could have been used.

In paper 1, the birth prevalence was reported as number of persons affected with CHD per 10,000 births (live births, stillbirths, and terminated pregnancies) for the entire period 1994-2009. Then, the prevalence of severe defects combined, VSD, ASD, and PDA were calculated by year of birth. Next, we modelled annual CHD prevalence with The National Cancer Institute´s Joinpoint Regression Program version 4.0.4(104, 105) in order to estimate annual per cent change (APC) with 95% confidence intervals, using the best fitting model. And finally, the yearly prevalence of severe defects was stratified on type of birth, i.e. live birth (singleton or multiple birth) with isolated severe heart defect, live birth with severe heart defect and extracardiac defect, stillbirth with severe heart defect, and terminated pregnancy with a foetus affected with severe heart defect. The median age at diagnosis was calculated for severe CHD, ASD, VSD, and PDA, for births in the period 1994-2007, allowing nearly 3 years follow-up. The data linkage and all calculations were performed with SAS (version 9.3; SAS Institute Inc., Cary, NC, USA).

In paper 2 and 3, the risk of CHD in the child was expressed as relative risks (RR), calculated as the risk of CHD in the exposed group divided by the risk of CHD among the unexposed. Crude and adjusted relative risks (aRR) with 95% confidence intervals (CI) were estimated using binomial log linear regression models with a log-link function using Stata

version 13 (paper 3) and 14 (paper 2) (Stata Corp., Texas, USA). The covariates considered as potential confounders of the association between the exposure and infant CHD risk in the multivariable models were chosen a priori.

In paper 2, analyses of CHD risk in offspring of diabetic women were compared to the risk on children of non-diabetic women, adjusted for year of birth, maternal age(<20, 20-24, 25-29, 30-34 and >34 years), and parity(0, 1 or ≥2 previous pregnancies). In addition, RRs of CHD for maternal diabetes type 1 vs. type 2 were compared using the suest (seemingly unrelated estimation) command, and time trend for CHD risk by year of birth by regression analyses.

In paper 3, births without maternal intake of folic acid or multivitamin supplements were used as reference group, and analyses were adjusted for year of birth, parity, mother´s age, family income (quartiles of mean income of the adults in the family), education (≤10, 11-13, 14-16, ≥17 years, missing), marital status (married/cohabitant, single/other), and smoking (regular/occasional, non-smokers, did not consent to register smoking information, missing).

3.6 Ethical considerations

We have received permission from the Scientific Ethics Committee (036.09; 19.02.2009), the Medical Birth Registry (08-1107/364; 15.4.08), the National Board of Health, the Data Protection Agency, the tax authorities (owner of the National Population Register), and from Oslo University Hospital (The Data Protection Officer and the Department of Pediatric Cardiology).

4. Summary of main results

4.1 Paper 1 Congenital heart defects in Norway

Most children with CHD were registered in several data sources: 37% in two sources, 15% in three sources, and 1% in four sources (this would apply only to deceased children). Among children with severe CHD 78% were registered in two or more registries (Fig. 2). When assigning births, infants, or children with CHD into cardiac phenotypes, diagnosis codes were first selected from BERTE, which had registered 48.0% of the children identified with CHD in this project, then the first entry in PAS (36.1%), MBRN (15.2%), and the Cause of Death Registry (0.7%). CHD only reported by one source were found evenly in the three main registries (in PAS 17%, BERTE 15%, and MBRN 15%), in addition 1% in the Cause of Death Registry. The severe CHD reported by one source were registered in MBRN in 10%, PAS in 7%, BERTE in 4%, and the Cause of Death Registry 1% of the cases. Among all children with any CHD and severe CHD, the proportion reported by BERTE was 48% and 74%, by PAS 66% and 78%, and by MBRN 50% and 61%, respectively.

Figure 2. CHD registration in 4 sources

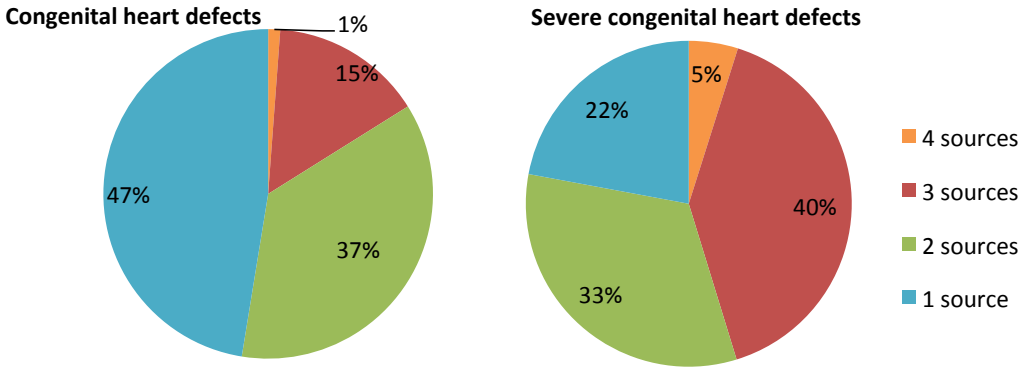


Figure 2. Individuals with CHD by the number of sources reporting any CHD or any severe CHD in each individual classified with CHD or severe CHD

Among 954,413 births, we identified 13,081 individuals with a congenital heart defect, yielding a birth prevalence of 137.1 per 10,000 births. The prevalences of CHD phenotypes per 10,000 births were: heterotaxia 1.6; conotruncal defects 11.6; atrioventricular septal defects 5.6; anomalous pulmonary venous return 1.1; left ventricular outflow tract obstructions 8.7; right ventricular outflow tract obstructions 5.6; septal defects 65.5, isolated ductus arteriosus (PDA) 24.6; and other specified or unspecified CHD 12.7. Excluding preterm PDA, the CHD prevalence was 123.4 per 10,000. The live birth prevalence of CHD was 133.2 per 10.000 and excluding preterm PDA 119.4 per 10,000.

Figure 3. Birth prevalence of CHD by year of birth

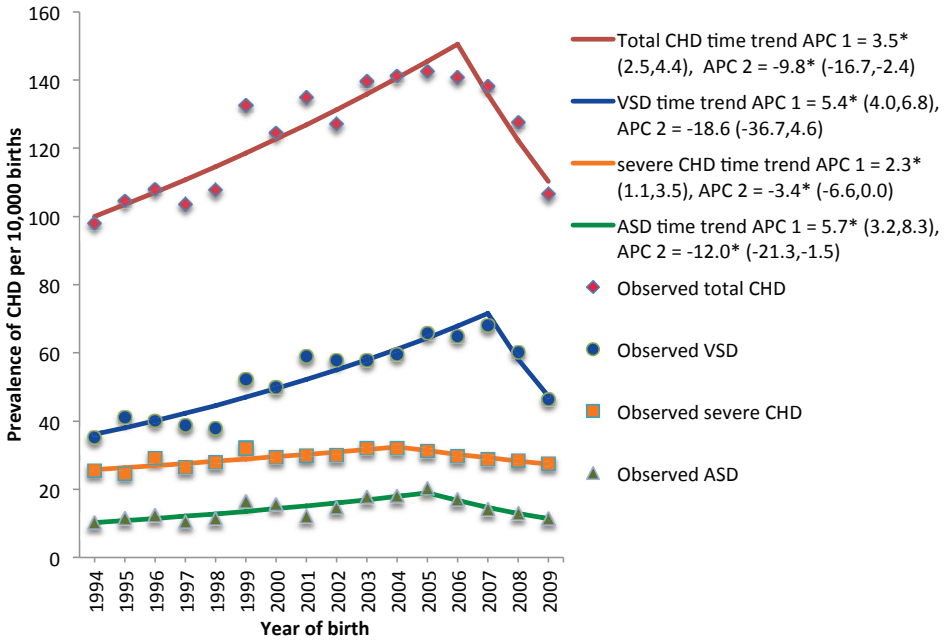


*Figure 3. Birth prevalence of CHD per 10,000 live births, stillbirths, terminated pregnancies by year of birth for all CHD (excluding isolated preterm PDA), VSD, ASD and severe heart defects in 954,413 births, Norway, 1994-2009. Observed and estimated prevalence, and Annual Percent Change (APC) with 95% confidence interval, using Joinpoint Regression Program. *p<0.05*

Severe CHD prevalence was 30.7 per 10,000 births. Both the prevalences of total CHD and severe CHD increased until around 2005, and decreased thereafter. Among all births, the prevalence of CHD increased with 3.5% (95% confidence interval 2.5, 4.4) per year in 1994-2005, and declined with 9.8% (-16.7, -2.4) per year from 2005 onwards. For severe CHD per year increase was 2.3% (1.1, 3.5) in 1994-2004, and per year decrease 3.4% (-6.6, -0.0) in 2004-2009 (Figure 3). These numbers included severe CHD in stillbirths and terminated pregnancies. Figure 4 shows the annual birth prevalence of severe CHD in combinations of birth type, plurality (singletons, multiples), and the presence of extracardiac birth defects and/or chromosomal aberrations.

Figure 4. Birth prevalence of severe CHD by year of birth

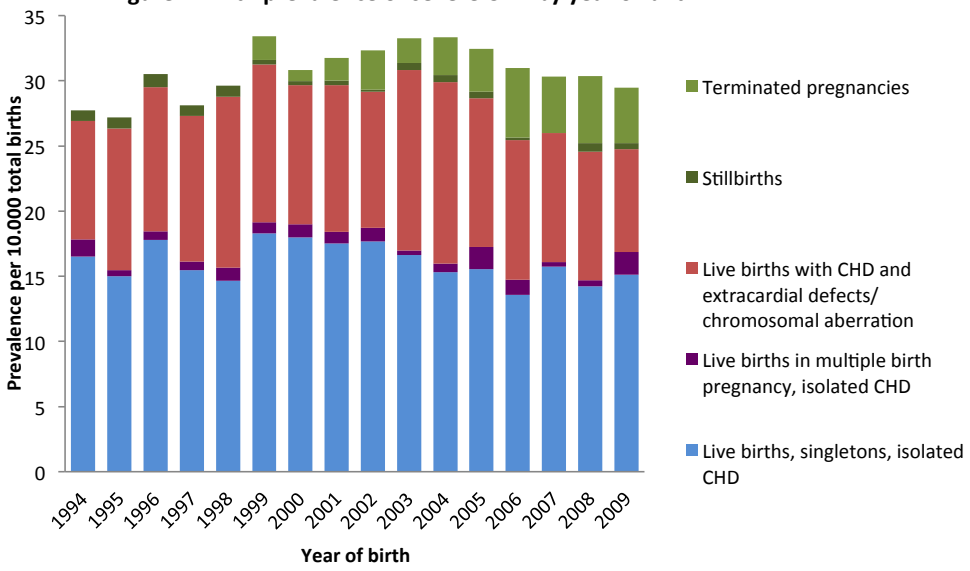


Figure 4. Birth prevalence of severe CHD by year of birth, stratified on type of birth (live birth, stillbirth/terminated pregnancies, singleton/multiple birth), and type of cardiac defects (isolated or accompanied with extracardiac birth defects and/or chromosomal aberration) in 954,413 births, Norway, 1994-2009.

4.2 Paper 2 Maternal diabetes and congenital heart defects

Among 914,427 singleton children without chromosomal disorders 1994-2009, 10,575 were registered with a diagnosis of CHD (116 per 10,000 births). Maternal pregestational diabetes (type 1, type 2, unspecified) was diagnosed in 5,618 (0.61%) and gestational diabetes in 9,726 (1.06%) of the pregnancies. While the prevalence of diabetes type 1 increased slightly from 42.4 per 10,000 births in 1999 to 47.3 in 2009, type 2 diabetes more than doubled in the period from 10.6 to 27.1 per 10,000 births, and pregestational diabetes increased accordingly from 72.2 in 1994, 84.9 in 1999 to 165.5 per 10,000 births in 2009 (Fig. 5).

Figure 5. Prevalences of maternal pregestational diabetes and gestational diabetes in 914,427 births in Norway, 1994-2009

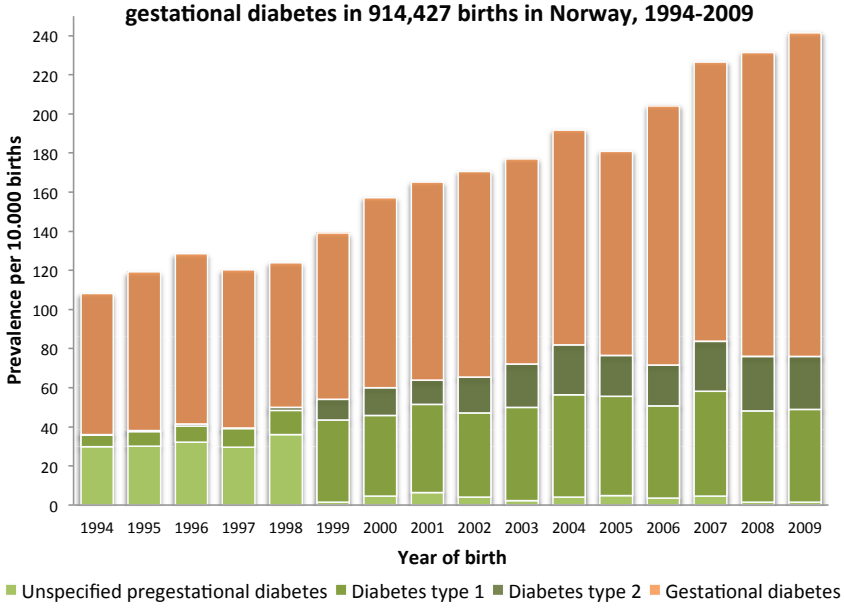


Figure 5. Prevalence of maternal diabetes mellitus by year of birth in 914,427 live births, stillbirths, and terminated pregnancies registered in the Medical Birth Registry of Norway, 1994-2009, excluding multiple births and children with chromosomal aberrations or genetic disorders.

Compared to offspring of women without diabetes, children of women with pregestational diabetes had a tripled risk for any CHD and severe CHD. Adjusted RR for any CHD was 2.86 (95% CI 2.50, 3.27), aRR for severe CHD 3.23 (95% CI 2.41, 4.34). There was no significant risk difference in type 1 and type 2 diabetes for any CHD (p=0.398) or severe CHD (p=0.212). Mothers with gestational diabetes had a 40% risk increase for having a child with CHD. Adjusted RR for any CHD was 1.43 (1.23, 1.65), for severe CHD a RR 1.40 (1.00, 1.96). There was no risk difference for CHD subgroups. In offspring of mothers with pregestational diabetes, RR for CHD showed a non-significant slightly decreasing trend (p=0.306), while there was no change in RR for CHD in pregnancies with gestational diabetes (p=0.693) (Fig. 6).

Figure 6. Relative risk for CHD by maternal pregestational diabetes and gestational diabetes in 914,427 births in Norway, 1994-2009

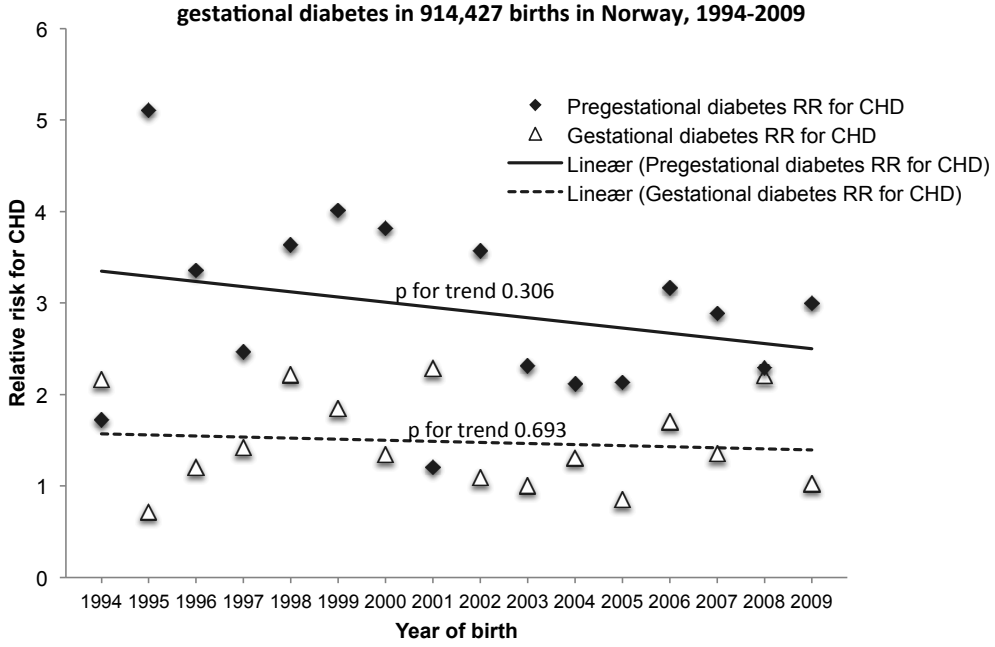


Figure 6. Adjusted relative risks for CHD (not included preterm PDA) by maternal pregestational diabetes, and gestational diabetes and by year of birth, in 914,427 live births, stillbirths, and terminated pregnancies registered in the Medical Birth Registry of Norway, 1994-2009, excluding multiple births and children with chromosomal or genetic disorders.

We investigated the association between birth weight and CHD risk in children born after gestational week 20, using infants with birth weight appropriate for gestational age (AGA) as reference group in each category. For children born LGA the risk for CHD increased by increasing birth weight in all groups. Among women with pregestational diabetes and gestational diabetes, offspring with weight >+3 SD more than the Norwegian mean had aRR for CHD 2.23 (95% CI 1.39, 3.59) and 2.73 (95% CI 1.53, 4.85) compared to newborn with AGA. Children with non-diabetic mothers born LGA >+3 SD had aRR 1.75 (95% CI 1.38, 2.22) compared to AGA infants.

4.3 Paper 3 Periconceptional folic acid and congenital heart defects

Among 517,784 singleton births without chromosomal disorders 1999-2009, 6,200 children were identified with CHD and 1,153 with severe CHD. Overall, 95,509 (18.4%) mothers had initiated folic acid supplements before pregnancy, 163,486 (31.6%) had started during pregnancy, 43,441 (8.4%) used only multivitamins in the periconception period, while 215,348 (41.6%) did not use any folic acid or multivitamin supplements (Table 5).

Table 5. Maternal use of folic acid or multivitamin supplements before and during pregnancy in 517,784 births, Medical Birth Registry of Norway, 1999-2009

		Supplement use during pregnancy			
		Folic acid		No folic acid	
Supplement use before pregnancy		Multivitamin No. (%)	No multivitamin No. (%)	Multivitamin No. (%)	No multivitamin No. (%)
Folic acid	Multivitamin	32,477 (6.3)	2,196 (0,4)	1,172 (0.2)	1,538 (0.3)
	No multivitamin	16,485 (3.2)	36,725 (7.1)	1,419 (0.3)	3,497 (0.7)
No folic acid	Multivitamin	18,539 (3.6)	2,572 (0.5)	12,496 (2.4)	1,986 (0.4)
	No multivitamin	62,170 (12.0)	80,205 (15.5)	28,959 (5.6)	215,348 (41.6)

Shorter education, younger age, smoking, previous births, single status, and lower family income were more frequent in mothers who did not use any folic acid or multivitamin supplements.

Initiation of folic acid before pregnancy increased from 5.5% of all births in 1999 to 29.1% in 2009, while any periconceptional use of folic acid increased from 21.7% in 1999 to 74.2% in 2009 (Fig. 7).

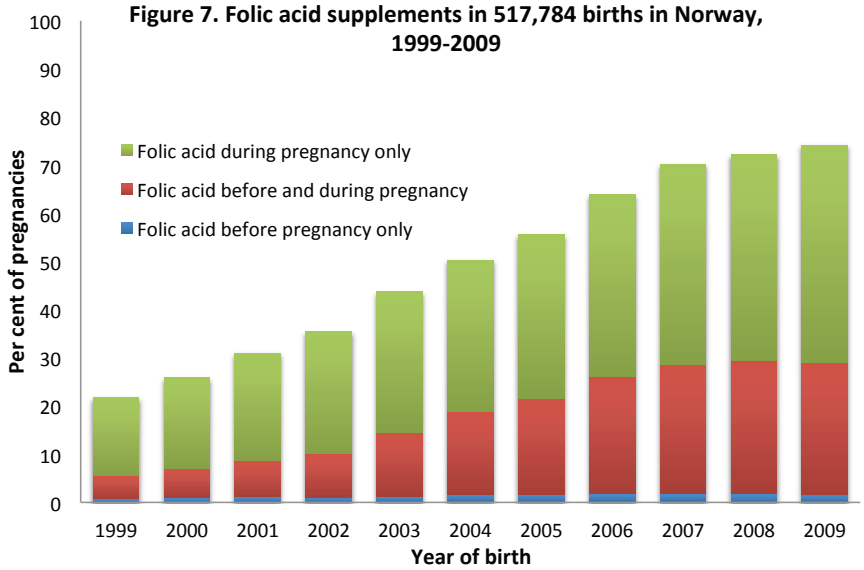


Figure 7. Periconceptional use of folic acid supplements by year of birth in 517,784 live births and stillbirths in Norway, 1999-2009. Births without information on maternal supplement use, or with chromosomal disorders, multiple births, from in vitro fertilisation, or with maternal epilepsy, were excluded.

Maternal periconceptional use of folic acid was not associated with infant risk of severe CHD; the aRR was 0.99 [95% CI 0.86, 1.13] comparing pregnancies exposed to folic acid with non-exposed pregnancies. Specifically, aRR for conotruncal defects was 0.99 (95% CI 0.80, 1.22),

aRR for AVSD 1.19 (95% CI 0.78, 1.81), aRR for LVOTO 1.02 (95% CI 0.78, 1.32), and aRR for RVOTO 0.97 (95% CI 0.72, 1.29). Surprisingly, septal defects (i.e. ASD, VSD, other septal defects) and term PDA were associated with a 20% increased risk in infants whose mothers used folic acid, as compared to nonusers. For preconceptional or postconceptional initiation of folic acid, the RRs were similar for severe defects (aRR 1.10 and aRR 0.97, respectively) and for septal defects (aRR 1.18 and aRR 1.19, respectively).

5. Discussion

5.1 Methodological considerations

5.1.1 Study design

Different study designs allow varying threats to the validity of the findings. A hierarchy of evidence is widely used to evaluate the quality of medical studies(106): first controlled randomised studies; then observational studies: cohort studies, case-control studies, cross-sectional surveys (ecological studies); and finally case reports. Because exposition groups are not randomly selected in observational studies, confounding is a potential threat to the internal validity

The present study was a national cohort study with linkage of data from national administrative registries and health registries. The study included all births in Norway 1994-2009 (live births, stillbirths, and terminated pregnancies for foetal abnormality registered in MBRN) with exposure information obtained from MBRN and PAS, and with outcome information, i.e. CHD retrieved from MBRN and PAS, 1994-2009, and BERTE and CDR, 1994-2010. This gives a high quality of evidence; however, a controlled randomised study design would have been even better to avoid confounding of the association between maternal folic acid supplement use and offspring CHD.

5.1.2 Case ascertainment and classification

Ascertainment of CHD cases through 4 comprehensive and compulsory administrative and clinical registries enabled a virtually complete registration of both severe and minor CHD detected in Norway during the years 1994-2009, including cardiac defects in terminated pregnancies. This assumption is substantiated by the high prevalences of CHD in our study, as compared to previous studies. This was possible due to the unique personal identification

number given shortly after birth, allowing follow-up through life. Since medical care for children is free in Norway, and health care highly developed, we expect nearly all children with CHD would have been diagnosed at an early age.

The cardiac phenotype hierarchy

Many previous studies have reported the prevalences of each cardiac defect rather than the number of affected individuals(107). The hierarchical structure of our classification system assigning individuals with multiple heart defects to only one cardiac phenotype precluded counting these individuals several times, thus avoiding an overestimation of the total prevalence of CHD. This system implies, on the other hand, underreporting of CHDs with the lower priority in this hierarchy (e.g. septal defects or PDA). Also, individuals with a combination of cardiac anomalies will be grouped with individuals affected by the CHD of highest priority, despite a different clinical picture and often a different prognosis. In case of several CHD diagnoses in one individual, the CHD phenotype was determined by the presumed first disruption in foetal cardiac development assuming the first error might influence later cardiac development. The classification of CHD is merely based on diagnoses and procedure codes available in the various registries, with no regard to the anatomical severity or the clinical impact of the cardiac malformation in each individual. Our classification system would therefore probably not be the most appropriate for studies on prognosis or treatment options of CHD.

However, the hierarchical classification system allowed analyses of the association of possible etiological factors and CHD phenotypes. In some cases, the phenotype assigned might not be the CHD with the greatest clinical relevance. However, according to my clinical experience the priority in our system corresponds relatively well with the severity. In addition, between cardiac defects assumed to occur at the same time, diagnoses considered more severe were prioritised highest.

Classification into cardiac phenotypes

In this large cohort study based on registry data the specific cardiac malformations could have been assigned incorrect diagnosis codes. Reviewing of the medical records for 13,000 patients to avoid misclassification of diagnosis codes would not be feasible. However, almost half of the cases and the majority of the severe defect cases were retrieved from BERTE by Van Mierop codes. Senior paediatric cardiologists have entered and updated codes for heart defect diagnoses and procedures into this clinical database at each patient consultation, which has minimised the possibility of misclassification of severe defects. Also, the detailed Van Mierop coding system allowed a high accuracy of the CHD classification. Furthermore, in PAS, we selected diagnosis codes from the university hospitals with facilities for paediatric cardiology surgery and invasive procedures, assuming that specialists had set the diagnoses from these hospitals. Finally, we crosschecked diagnosis codes in all 2999 individuals notified with conditions considered lethal without intervention against their survival status at age 2 years and surgery or procedure codes, and identified only 89 (2.8%) likely misclassified severe defect cases, which were put in the unspecified CHD category.

BERTE contains a key converting Van Mierop codes to ICD-10 codes. However, systematic errors and inaccuracies were discovered in this conversion system, and we therefore chose to give ICD-10 codes from BERTE low priority.

Missed diagnoses

Diagnoses of CHD in individuals with chromosomal aberrations and/or extracardiac defects could have been missed in case the cardiac defect was not coded, for example in stillbirths or terminated pregnancies. The registered prevalence of CHD in births with Down syndrome was lower in terminated pregnancies than live births, suggesting an underreporting of CHD in these pregnancies. However, the proportions of chromosomal aberrations (10.3%) and extra-cardiac defects (20.6%) in children with CHD were comparable to findings from Denmark (7.0% and 22.3%) (19).

Physiological heart conditions

Ductus arteriosus is an important structure in foetal life, allowing the blood to bypass the developing lungs. This duct normally closes during the first few days after birth, and is considered a heart defect only if persistent after the neonatal period. Likewise, an opening between the left and right atria necessary in the foetal circulation closes by adapting of septum primum and septum secundum shortly after birth, but an intermittent shunt may still be visible the first weeks after birth. There was a risk of including these physiological heart conditions, which might have been coded as PDA or ASD. A physiological transient peripheral pulmonary stenosis may also lead to overdiagnosing of pulmonary stenosis in newborn children. To avoid this, untreated ASD, PDA and vPS only recorded the first 6 postnatal weeks were excluded. Due to this exclusion and the fact that children with minor CHDs may be seen in the outpatient clinics only, some minor CHD could have been missed. Outpatient clinic data was only included from Oslo University Hospital by BERTE. Considering the high prevalence of minor heart defects in the present study, as compared with previous studies, we considered the loss of minor defects only followed in the remaining outpatient clinics of less importance.

Preliminary identification numbers

All newborn children are given a temporary identification number at birth by the birth institution, pending the life-long personal identification number, which is assigned after a few days. In some cases, children might have duplicate records in the hospitals, if records registered with the two numbers were not linked. In our data set, only live born children with valid permanent identification numbers were included. Therefore, diagnoses indicating a CHD only available in medical records registered with a temporary number could have been missed. This would, however, apply only to minor defects followed in the outpatient clinic. Severe CHD would come to attention later because of follow-up, treatment, surgery, procedure, or death.

5.1.3 Definition of Diabetes Mellitus

Information on diabetes mellitus was collected from MBRN by yes/no variables and ICD codes, and from PAS by ICD codes. A validation study (108) of the diabetes diagnoses in MBRN, comparing the variable with the Norwegian Diabetes Registry(109), showed very good sensitivity of 97% for pregestational diabetes before 1999, and 94% in the years 2000-2004. Positive predictive value was examined by conferring medical records for pregnant women who gave birth in 1998 and was 80% for pregestational diabetes (half of the incorrect diagnoses were really gestational diabetes). Gestational diabetes was confirmed in 89% of cases. Information of maternal diabetes was also collected from the PAS database, which contributed 171 additional cases of pregestational diabetes (3.0% of mothers with pregestational diabetes) and 1772 (22.3%) additional cases of gestational diabetes.

Analyses of type 1 and type 2 diabetes were restricted to the period 1999-2009. Until 1998, the notification form from MBRN had one check box for pregestational diabetes. In 1994-1998, diagnoses of type 1 diabetes or type 2 diabetes in some cases were specified in MBRN by ICD codes, and a few additional diagnoses were identified by the PAS data. These were, however, sparse. In the period 1994-1998 totally 224 mothers were notified with type 1 diabetes in MBRN, and additional 30 in PAS (18.8% of 1191 women with pregestational diabetes), and only 17 + 3 mothers were registered with type 2 diabetes (1.7%). Among 4427 mothers with pregestational diabetes in the period 1999-2009 type 1 diabetes was diagnosed in 2931(66.2%), type 2 diabetes in 1278 (28.9%), and unspecified pregestational diabetes in 218 (4.9%).

While the prevalence of type 1 diabetes was relatively stable throughout the study, the prevalences of diabetes type 2 and pregestational diabetes increased substantially from 1999 to 2009. The diagnostic criteria for diabetes as defined by WHO were revised in 1998 with a lowered diagnostic cut-off level for fasting plasma glucose from ≥7.8 mmol/l to ≥7.0 mmol/l(35). The cut-off levels for random glucose and glucose load test (both 11.1 mmol/l) were not changed. The diagnostic criteria for gestational diabetes were also extended to

include impaired glucose tolerance with cut-off for plasma glucose ≥7.8 mmol/l after a glucose load test, as well as the lowered fasting glucose level of ≥7.0 mmol/l. However, these diagnostic changes had no clear influence on the time trend of maternal diabetes. The prevalences of both pregestational diabetes and gestational diabetes were continuously increasing after 1998. Whether an improved screening of diabetes in women before and during pregnancy has contributed to this cannot be ruled out.

5.1.4 Folic acid and multivitamin supplements

From 1999, registration of periconceptional folic acid and multivitamin supplement use has been mandatory in Norway and notified by check boxes in MBRN. This allows population-based analyses of associations between supplement use and various pregnancy outcomes. The exposure variable, however, has certain weaknesses, such as underreporting of supplement intake, and lack of information on timing and dosis of folic acid supplement intake. At present, there has been no validation study of the variable. Estimates of sensitivity have, however, been performed for the first years of the study period by comparing folic acid supplement intake registered at the birth notification form with information on reported folic acid intake among mothers participating in the Norwegian Mother and Child Cohort Study (www.fhi.no). Among 9,407 participants of the Norwegian Mother and Child Cohort Study 1998-2001, 6.3% reported use of folic acid supplements. Of these, 45% were registered as nonusers in MBRN (110). This period is, however, not representative for the study period, as the compliance with the new registration form increased in many birth clinics the first years. An underreporting of folic acid use will most likely bias the association measures towards the null. However, the estimates of relative risk for any CHD or severe CHD in offspring of women reporting use of folic acid or multivitamin supplements were similar for the period 1999-2004 and 2005-2009, suggesting that underreporting of folic acid use in the beginning of the study period less likely affected relative risk estimates.

MBRN does not register timing of supplement use initiated during pregnancy. Folic acid or multivitamin supplement use after the first trimester of pregnancy has probably little impact on cardiac development, because the foetal heart is fully developed by the 10^{th} week of pregnancy. However, in more than 85,000 pregnancies included in the Norwegian Mother and Child Cohort Study 2001-2008(111) almost all folic acid users reported initiation of supplementation at gestational week 4 to 5, corresponding to 2 to 3 weeks post conception. The participants of the Mother and Child Cohort Study are self-selected and the findings in this group may not be fully generalizable to the rest of the population, but it is reasonable to assume that the majority of women notified with supplement use during pregnancy had initiated this in the period of cardiac development (3-7 weeks post conception). This assumption is supported by consistent findings for initiation before or during pregnancy of the associations between folic acid or multivitamin supplement use and CHD.

Finally, MBRN contains no information on folic acid dose in folic acid or multivitamin supplements. However, the only folic acid tablets sold in Norway during the study period contained the recommended daily dose of 0.4 mg folic acid, and the folic acid content in multivitamins ranged 0.1 - 0.4 mg.

5.1.5 Bias and confounding factors

Bias is a systematic error in the methodology of a study, resulting in incorrect estimates or interpretation of the association between an exposure and the outcome. Bias can be classified in three categories: selection bias, information bias, and confounding(112, 113).

Selection bias

Selection bias refers to an error introduced by factors influencing the selection of study subjects or the participation in the study, resulting in a study population that is not representative of the target population. The study populations in our project were national cohorts with compulsory participation through MBRN, and the life-long personal identification-number given to every Norwegian resident enables a very low risk of loss to follow-up. The risk

of selection bias was therefore small. Possible sources for selection bias include exclusion of children with missing information on maternal health (paper 2); birth weight or gestational age (paper 2); and mother´s use of folic acid or multivitamin supplements (paper 3).

Paper 2. Maternal diabetes and congenital heart defects

Only 0.4% had missing information on maternal health in paper 2, and this was unlikely to introduce bias in the estimates of the association between maternal diabetes and offspring risk of CHD. Z-score for birth weight by gestational age was missing in 15.4%. There was no difference between the children with missing information on birth weight z-score and the rest of the population regarding maternal pregestational diabetes (0.6% in both groups) or GDM (1.0% and 1.1%). The prevalence of CHD and especially severe CHD was higher among excluded births (1.7% vs. 1.1% for all CHD and 0.67% vs. 0.22% for severe CHD). This is partly caused by missing information on birth weight for all terminated pregnancies. After excluding terminated pregnancies (1,237 pregnancies; 205 with CHD, 142 with severe CHD) the prevalences of CHD and severe CHD in the group with missing information on gestational age or birth weight were almost similar to the rest of the population (1.2% and 0.3%). It is therefore not likely that the selection of children with information on birth weight z-score influences the estimates of the association between high birth weight and CHD.

Paper 3. Periconceptional folic acid and congenital heart defects

In the study of the association between maternal use of folic acid supplements and offspring CHD information on folic acid or multivitamin supplement use was missing in 15% of all births. Among these, 15.4% were pregnancies terminated for foetal reasons. The terminated pregnancies amounted to only 0.2% of all births, and sensitivity analyses (counting all terminated pregnancies in either the exposed or the unexposed group) showed no impact of supplement use in this group on the estimates of relative risk except for hypoplastic left heart syndrome, which to a large extent were terminated. The prevalence of CHD was increased in all terminated pregnancies (144 per 1,000), but the proportion of terminated pregnancies among all births with any CHD (2.7%) or severe CHD (9.3%) was low, and amounted to a

relatively small part of the births with CHD. Overall, the prevalences of severe defects and septal defects were similar among births with missing folic acid information and the study population. Maternal characteristics (education, age, parity, family income, smoking) in the group with missing information on folic acid supplement use were similar to the general population. A systematic difference between the pregnancies with missing information on supplement use and the population in general with respect to folic acid intake is therefore not likely.

Information bias

Information bias refers to misclassification of the study subjects for either the exposure or the outcome. The misclassification can be differential (if the error in information of exposure is related to the outcome or vice versa) or non-differential. While differential misclassification can either overestimate or underestimate an effect, non-differential misclassification tend to weaken the association of the exposure and the outcome.

Misclassification of outcome (paper 1-3): Diagnoses from outpatient consultations were not available in PAS, but reported in BERTE, which could lead to an overrepresentation of minor CHD from the Oslo area (detection bias). Despite a highly developed health care for children in Norway, there are large distances to specialist service in some parts of the country, and the threshold for referring a probable innocent murmur may vary. This might lead to a lower detection of minor CHD in rural areas. However, the possible referring differences are probably mainly applicable to outpatient consultations. As only diagnoses from hospital stays were included in PAS, it is less likely that varying referral practices causes bias. It is, however, likely that children born preterm or with extra-cardiac malformations or perinatal diseases, could have been especially thoroughly investigated shortly after birth. This might imply increased detection of minor CHD, but probably not of severe CHD. CHD diagnoses could, on the other hand, be registered to a lesser extent in pregnancies terminated for other reasons (e.g. chromosomal disorders). The prevalence of the septal defects could have been underestimated in the last period due to shorter follow-up time, because these defects are

likely to be diagnosed later in infancy(19), supported by our finding that the median age at diagnosis for ASD was 337 days. However, severe CHD, and also VSD were to a large extent diagnosed at the maternity ward, and 94% of severe CHD and 97% of VSD were registered with diagnosis date before 6 months of age. Therefore, the declining prevalence of severe CHD from 2005 through 2009 cannot be explained by incomplete ascertainment of severe cases due to a shorter follow-up of births in the late period.

Paper 2: Since maternal diabetes increases the risk of preterm birth as well as other pregnancy complications(114), the newborn of diabetic women might be more likely to undergo cardiac examinations with subsequent increased detection of minor CHD like ASD or PDA. This could cause a differential misclassification. However, the relative risk of severe CHD, which are almost always diagnosed shortly after birth, was somewhat stronger than the relative risk of septal defects in children of diabetic women as compared to non-diabetic women. Detection bias does therefore not seem to affect the results.

Paper 3: There has been underreporting of folic acid supplement use in MBRN in the first part of the study (110). The underreporting is most likely non-differential and related to several birth clinics failing to report the new information required in the revised birth notification form from 1999. This could weaken the association measures and, in theory, conceal an association between folic acid intake and overall CHD. The information regarding supplement use will usually be filled in the notification form during pregnancy; i.e. in most cases prospectively with regard to detection of CHD in the child. However, in some women, this information might be completed during the hospital stay at time of delivery. An increased awareness of details as periconceptional supplement use in mothers of children with a CHD cannot be ruled out. This could possibly bias the results towards either a positive or a negative association between supplement use and CHD.

Confounding factors

Confounding can be defined as the confusion of effects(113). A confounder is a factor independently associated with both the exposure under study and the outcome (i.e. a risk factor of the outcome). The effect of the exposure might be mixed with the effect of the confounding variable, leading to misinterpretation of the findings. To control for the potential confounding factors, the study population may be restricted or the observed association can be adjusted for in the analyses. In paper 2 and 3, potential confounders were chosen *a priori*, and evaluated according to their effect on the estimates of relative risk.

Paper 2: The *a priori* selected confounders for the association between maternal diabetes and CHD were year of birth, maternal age and parity, maternal education and family income. The socioeconomic status of the mother is, however, closely related to the risk of overweight and diabetes(115), and because this might partly cause the possible effect on risk for CHD, adjusting for education and income was considered likely introducing overadjustment. The prevalences of both CHD and maternal diabetes varied considerably during the study period. Maternal age and parity are individual risk factors for CHD(29, 116) and also for maternal diabetes(117). Year of birth, maternal age, and parity were included as confounders in the final model. The adjustments did not change the overall estimates of relative risk, so residual confounding was less likely. The results were not considered affected by confounding.

Paper 3: Potential confounders of the association between intake of folic acid and infant CHD risk are year of birth, maternal age and parity, maternal education and family income, marital status, maternal smoking, maternal diseases like diabetes or epilepsy, and conditions related to the pregnancy like in vitro fertilisation (ivf) or multiple birth pregnancies. Multiple births and ivf pregnancies have increased risk for CHD and are strongly associated with increased use of folic acid supplements(110). Anti-epileptic medication (e.g. sodium valproate, carbamazepine) interferes with the folate metabolism. Women with maternal epilepsy have increased risk for congenital malformations, and are recommended high doses of periconceptional folic acid supplementation; 4-5 mg daily(118). Multiple births, births from ivf, and with maternal

epilepsy were considerably different from the rest of the cohort and we decided to control for these potential confounders by restriction of the analyses to singleton births without ivf or maternal epilepsy. In the study population the mother was diagnosed with pregestational diabetes mellitus in 3517 pregnancies (6.8 per 1,000). Initially, we restricted the analyses to births of non-diabetic women, but the results did not differ from the main analyses including births with maternal diabetes, with respect to any use of folic acid and for pregestational use of folic acid. We therefore decided to include births with maternal diabetes.

Periconceptional intake of folic acid supplements had increased during the study period, while the birth prevalence of CHD was varying. Maternal age, parity, socioeconomic factors (maternal education, income, marital status), and maternal smoking are possible risk factors for CHD(33, 119), and also predictors for maternal use of folic acid supplements(120). Year of birth, parity, family income, mother´s age, education, marital status, and smoking were included as confounders in the final model. The adjustments had little influence on the overall estimates of relative risk. We did not identify possible unmeasured confounding factors, but unknown residual confounding cannot be ruled out as explanation of the unexpected association of folic acid supplementation and an increased risk of septal defects.

5.2 Discussion of the main results

5.2.1 Prevalences and time trends of congenital heart defects

The prevalence of CHD in the present study was higher than reported in similar studies from Denmark, other European countries, and Atlanta, USA(19, 105, 121). In the Danish study(19), the overall prevalence excluding isolated PDA in preterm infants was 103.2 per 10,000 live births, versus 123.4 in our study. It is, however, primarily the prevalence of septal defects, particularly VSD that was higher in the present study. While studies from the EUROCAT registries (121) and Atlanta(105) reported considerably lower prevalence of the severe CHD as

compared to the present study, the prevalences of severe defects in Denmark and Canada were similar to our findings(19, 22). This is most likely due to a nearly complete detection of minor CHD in our study, which was possible by combining national registries and large databases.

Although the severe CHD prevalence was lower in the EUROCAT study, the pattern of time trends was similar to the present study with an increase from 1990 to 2004, and a decrease thereafter. The Danish study also reported an increasing prevalence of severe heart defects from 1977 to 2005, like the present study. As suggested in the previous studies(19, 21, 105), the increasing prevalence of minor CHD may be partly explained by improved diagnostic equipment. This applies primarily to the period before year 2000. The continued increase of both minor and severe CHD until 2006, however, has most likely other explanations. We cannot rule out that the CHD prevalence increase could partially be explained by improved reporting of birth defects. In 1999 MBRN implemented a revised notification form, which may have led to higher quality of birth defect reporting, including both mild and severe CHD. Mandatory reporting to MBRN of terminated pregnancies was implemented; from 1999 to 2001 for pregnancies after 16th gestational week, from 2002 after 12th gestational week(93). This might explain part of the increase in severe CHD from 1994 to 2004.

As mentioned previously, the declining prevalence of severe CHD from 2005 is less likely explained by short follow-up time in the late period. However, CHD diagnoses might be registered to a lesser extent in pregnancies terminated for other reasons (e.g. chromosomal disorders) resulting in an overrated declining trend of severe defects. Excluding all terminated pregnancies and offspring with extra-cardiac malformations or chromosomal aberrations gives a weaker declining trend of severe CHD from 2002, which might be explained by more available perinatal diagnostics and subsequent termination of pregnancy if severe CHD were detected.

5.2.2 The impact of maternal diabetes on prevalences of CHD

Maternal diabetes is the most important risk factor for CHD among other congenital malformations, the hyperglycaemia itself believed to be the major teratogen. Guidelines for pregnancy care have therefore emphasised the importance of preconception counselling and good glycaemic control throughout the pregnancy for women with pregestational or gestational diabetes, with the assumption that perfectly regulated diabetes in pregnancy normalises risk in offspring (122). The pregnancy care in Norway is highly developed and free for all women, and provides opportunity for optimised care for women with pregestational or gestational diabetes. We therefore analysed the risk of CHD in offspring of diabetic mothers in the Norwegian population, compared to children of non-diabetic women, with the expectation that the relative risk might be lower than reported from countries with a less developed public health system. Also, we wanted to investigate if the time trends of diabetes prevalences or a changing relative risk could explain the time trends of CHD in the population reported in paper 1. The risk increase in offspring of women with pregestational diabetes in our study was, however, similar to a recent European study; Macintosh et al described a 3.4 fold risk for CHD in 2359 pregnancies with pregestational diabetes in a cohort-study from England, Wales and Northern Ireland 2002-2003, similar for type 1 and type 2 diabetes(51). A population-based Canadian study of almost 2.3 million children born 2002-2010 showed a slightly higher risk for CHD with odds ratio of 4.65 in type 1 diabetes, and 4.12 in type 2 diabetes(123), similar to the findings in an American multicenter case-control study with children born 1997-2003, and odds ratio for CHD in pregestational diabetes of 4.64(124). This study by Correa et al also reported an almost 60% increased risk for CHD in pregnancies with gestational diabetes, compared to a 40% increase in our study.

While the prevalence of maternal diabetes type 1 was relatively stable, the increase of type 2 diabetes and gestational diabetes in the period 1999-2009 had epidemic proportions similar to previous findings from England, USA, and Canada(41, 124, 125). The increasing prevalence of diabetes could therefore explain some of the increasing prevalence of CHD until

2005, but while there was a continuously increase in prevalences of maternal pregestational diabetes and gestational diabetes until 2009, the CHD prevalence flattened and possibly decreased after 2005. This ecological approach, however, gives no definitive understanding of the association between maternal diabetes and offspring CHD, and individual information is necessary to determine the possible change in relative risk in this group. There was no significant reduction in relative risk for CHD in offspring of diabetic mothers in the period 1994-2009. This suggests a change in other important risk factors.

We did not have information on maternal use of insulin or oral antidiabetic medication during pregnancy, and could therefore not investigate the association between use of medication and risk of offspring CHD. However, previous studies have not found evidence of teratogenic effects of oral antidiabetic medication(126) or insulin(127, 128).

Information on glycaemic control during pregnancy by HbA1c or fasting glucose measurements was not available in the current study. However, the birth weight of the neonate could indicate glycaemic control during the pregnancy(64, 129, 130). We therefore chose to investigate birth weight of the offspring, with LGA as a marker for maternal hyperglycaemia. Although most heart defects develop during the first weeks, while birth weight may be influenced by hyperglycaemia later in pregnancy, poor glycaemic control causing macrosomia could be an indicator of hyperglycaemia in early pregnancy. The risk of all CHD and severe CHD increased significantly with increasing birth weight in offspring of mothers with both pregestational diabetes and gestational diabetes, supporting the theory that the maternal glucose level is the most important risk factor for CHD in diabetic pregnancies. Surprisingly, the risk of CHD increased similarly in LGA children of women without a diagnosis of diabetes. This could be caused by unrecognized or otherwise misclassified maternal diabetes in this group, by impaired maternal glucose tolerance not meeting the criteria for diabetes, or by high sugar intake resulting in hyperglycaemic spikes during pregnancy. A periconceptional sugar rich diet has been associated with an increased risk for several birth defects in offspring of non-diabetic women in a recent study(131). Other common

risk factor for macrosomia and CHD cannot, however, be ruled out, and the aetiology of CHD in children LGA may be different in diabetic and non-diabetic pregnancies.

5.2.3 Folic acid supplementation use and congenital heart defects

The possibly decreasing prevalence of both severe and non-severe CHD in Norway, as in other European countries from mid-2000s(121, 132) has been suggested explained by the increasing intake of periconceptional folic acid and multivitamins from 1999 to 2009(89). In Quebec, Canada, a distinct decreasing prevalence of severe CHD from 1999 coincided with implementation of folic acid fortification of grain products from 1998(22). Godwin et al found, however, no decrease in total CHD in Alberta, Canada, in a 5-year period post fortification (1999-2003) compared to the pre-fortification period (1992-1996)(83). In this study a decrease in prevalence of ASD was suggested, but in a recent study including information from several registries a 42% increase of ASD and 52% increase of VSD was described in the years after folic acid food fortification in Alberta, Canada(133). This study found a decline in prevalence of LVOTO (mainly CoA) in the post folic acid fortification period, but no change in the overall CHD prevalence. In Atlanta, Georgia, who led a similar food fortification policy, no reduction of CHD was found(105). As correlating time trends in populations do not necessarily show a causal relationship, studies with individual information of periconceptional folic acid supplementation and offspring CHD was necessary to determine a possible protective effect of folic acid supplementation.

Only a few previous studies have reported individual level information of maternal folic acid supplement use and infant risk of CHD. A Californian case-control study from 1995(80), reported reduced risk for conotruncal heart defects in children of mothers who had taken multivitamins or folic acid fortified cereals. A registry-based case-control study from the Northern Netherlands 1996-2005(82), reported a reduced risk for CHD, mainly septal defects, in offspring of women using periconceptional folic acid supplements. In these studies, the folic acid content was similar to the presumed dose in our study (0.4 mg/d). In a case-control study

from Hungary(134), however, much higher doses were used; the estimated average dose was 5.6 mg/d. This Hungarian study reported a reduced risk for conotruncal heart defect in the group exposed for folic acid supplements. In a Hungarian randomised controlled trial(81), multivitamin supplements with 0.8 mg folic acid were compared to supplements with other trace elements. There was significantly reduced risk for CHD in the group receiving vitamins with high-dose folic acid, but the numbers were small, with only 10 vs.20 CHD cases in the exposed and unexposed group, respectively.

Our population-based study showed no preventive effect of maternal intake of folic acid supplements on infant risk of severe CHD. For septal defects, there was approximately 20% increased risk in children whose mothers had taken periconceptional folic acid supplements. A possible risk reduction of CHD by intake of folic acid or multivitamin supplements is likely determined by the extent of dietary vitamin insufficiency in the population. The dietary folate intake reported in pregnant Norwegian women, around 300 microgram per day(135), could be sufficient for foetal cardiac development, as opposed to certain Chinese provinces with a high prevalence of folate deficiency, where the risk of CHD has been significantly reduced when mothers had used periconceptional folic acid supplements(136). Alternatively, the dose of folic acid supplement in the present study, 400 microgram per day, is too small to prevent cardiac malformations, although plasma folate has been found to be significantly higher in women reporting folic acid supplement use(137). However, no risk reduction was found, even in the group with both folic acid and multivitamin supplements before and during pregnancy (approximately folic acid dose of 0.6 mg/d), suggesting the use of folic acid supplements does not prevent CHD in the Norwegian population.

5.2.4 Possible adverse outcome of folic acid supplements

To our surprise, we found a significantly increased risk for both ASD and VSD when the mother had used folic acid supplements in the periconceptional period, which has not been reported

previously. The increased risk of septal defects was not modified by year of birth, maternal education, or prematurity. The positive association between folic acid supplements and risk of septal defects in the present study is unlikely a chance finding, but could be caused by an unknown residual confounding. A biological factor cannot, however, be ruled out.

While there is evidence that maternal periconceptional folic acid supplementation decreases the risk for some birth defects, which indicates that folate deficiency in pregnant women affects foetal development, there is some concern regarding the possible adverse effects of high intake of folic acid. During embryonic development, the genome undergoes reprogramming of the DNA, mediated by epigenetic modifications such as folate-dependent DNA methylation(138). In studies of pregnant mice a moderate to high intake of folic acid had adverse effects on offspring cardiac development. Mikael et al found that mice fed with a diet containing 10 times the recommended rodent folic acid intake showed an increased risk for VSD, and thinner ventricular walls than the control group(139). Another mouse model with mice fed on a folic acid-supplemented diet with a 20-fold increased folic acid amount compared to the recommended rodent intake showed similar adverse effects on offspring cardiac development with growth retardation and thinner ventricular wall(140). Though a diet with high folate content has been associated with low cancer risk, it has been suggested that higher intake of folic acid might promote growth of certain tumours(141). However, in two recent Norwegian population-based studies, use of periconceptional folic acid supplementation did not give increased risk of short-term maternal cancer or childhood cancer in offspring(142, 143).

6. Implications and future aspects

In this project we have developed a method for ascertainment of individuals with cardiac defects from several data sources with multiple coding schemes (ICD, versions 8, 9, 10; Van Mierop; procedure/surgery schemes), and established a key to classify these individuals with cardiac defects into cardiac phenotypes. The method is easily adapted to updated data sources and has also been applied to Danish register data (personal communication, Øyen N).

The birth prevalence of CHD in Norway was decreasing from 2005 until 2009, also when stillbirths and terminated pregnancies were counted. This decrease may be overestimated due to underreporting of CHD among pregnancies terminated with other foetal indication. The decrease is measured over a few years, and may be a fluctuation in prevalence rather than a lasting decline. The prevalences of CHD reported from MBRN to the EUROCAT network did not change from 2009-2011(144), but an update including all data sources is necessary to determine if the CHD decline has continued after 2009. New findings can be presented from the updated CVDNOR project through 2014, with information on all births from MBRN, 1994 through 2014, updated with information on CHD from PAS 2010-2014, the Norwegian Patient Register 2008-2014, and the Cause of Death Registry.

We would have expected an increase in CHD prevalence because of increasing prevalence of obesity, diabetes type 2, and increasing maternal age at delivery in the population. These risk factors for CHD could have been outweighed by the effects of better prenatal care at a population level, for instance an optimal glucose regulation around conception. We did, however, not find any significant change in CHD relative risk among offspring of diabetic women during the study period as compared to children of nondiabetic women. We chose to investigate macrosomia as a marker for dysregulated diabetes, as an effect modifier of the association between pregestational diabetes and offspring risk of CHD.

Previous studies have reported the association of mid-pregnancy glucose and risk of offspring CHD(145). Future observational studies of CHD and other birth defects in diabetic

women would benefit from incorporating glucose measurements prior to conception or in early pregnancy to evaluate the role of glucose in the causal pathway. Alternatively, pre-gestational diabetes may be associated with adverse foetal cardiac development independent of prenatal care. We found an increased risk for CHD in offspring with very high birth weight as compared to AGA children in both diabetic and non-diabetic women. We found increased risk of CHD in offspring very LGA compared to AGA infants of non-diabetic mothers. This corresponds to recent findings of an association between high periconceptional sugar intake(131) or high non-fasting mid-term blood sugar(145) in non-diabetic women. Further research is needed to evaluate high sugar intake during pregnancy as a risk factor for offspring CHD.

Brodwall et al(146) and Auger et al(147) have recently described an association between preeclampsia and CHD, hypothesised to be caused by a common etiological factor, for instance disturbance of angiogenic factors. Hyperglycaemia is known to cause endothelial dysfunction(148) and maternal diabetes is a risk factor for preeclampsia. Further studies are needed to investigate the etiological pathways between diabetes, preeclampsia and offspring CHD.

MBRN is the only population-based registry with information on periconceptional intake of folic acid supplements. Our findings of no association between periconceptional folic acid supplementation and decreased offspring CHD risk could be confirmed by a study including participants of the Mother and Child Cohort study, which also provides information on folic acid intake by week of gestation. Mandatory folic acid food fortification has been implemented in more than 70 countries worldwide (e.g. Canada, USA, and Australia), and implementation in European countries is an on-going discussion. Possibly harmful effects of increased folic acid levels in the general population must be taken into consideration. Our findings do not support food fortification. Folic acid supplementation is, however, likely to have a preventive effect on CHD in populations with severe dietary folate insufficiency, and our findings can only be generalized to populations with a similar dietary folate content as the Norwegian.

7. Conclusions

In conclusion, we found a decreasing prevalence of both severe and minor CHD in Norway after 2005 after an increase in the period 1994-2004. This corresponds to recent reports of decreasing prevalences in Europe and Canada. Studies investigating the most important established risk factor for birth defects; maternal diabetes, and a recently proposed protective factor; folic acid supplementation, did not explain the downward time trend. We found no indication of preventive effect of periconceptional folic acid supplements on CHD in Norwegian population, and possibly adverse outcome with increased prevalence of septal defects.

The prevalence of both pregestational diabetes and gestational diabetes was increasing in Norway 1994-2009. Despite efforts to improve perinatal care for women with pregestational or gestational diabetes(149), and reports of improvements in indicators of maternal care in Europe(41), we found no significant reduction in relative risk for CHD during the study period. An association between increasing birth weight and risk of CHD in our study supports the hypothesis that maternal hyperglycaemia is an important cause of embryopathy. Our findings suggest that modern periconceptional care is still not sufficient to reduce the perinatal risk associated with maternal diabetes.

Reference list

1. Mitchell SC, Korones SB, Berendes HW. Congenital heart disease in 56,109 births. Incidence and natural history. Circulation. 1971;43(3):323-32.
2. Kirby ML. Cardiac development. Oxford: Oxford University Press; 2007. xiii, 273 p. p.
3. Moorman A, Webb S, Brown NA, Lamers W, Anderson RH. Development of the heart: (1) formation of the cardiac chambers and arterial trunks. Heart. 2003;89(7):806-14.
4. Manner J, Wessel A, Yelbuz TM. How does the tubular embryonic heart work? Looking for the physical mechanism generating unidirectional blood flow in the valveless embryonic heart tube. Dev Dyn. 2010;239(4):1035-46.
5. Kiserud T, Acharya G. The fetal circulation. Prenat Diagn. 2004;24(13):1049-59.
6. Martinsen BJ, Lohr JL. Handbook of Cardiac Anatomy, Physiology, and Devices2005.
7. Bertrand N, Roux M, Ryckebusch L, Niederreither K, Dolle P, Moon A, et al. Hox genes define distinct progenitor sub-domains within the second heart field. Dev Biol. 2011;353(2):266-74.
8. O'Rahilly R, Muller F. The development of the neural crest in the human. J Anat. 2007;211(3):335-51.
9. Gittenberger-de Groot AC, Bartelings MM, Deruiter MC, Poelmann RE. Basics of cardiac development for the understanding of congenital heart malformations. Pediatr Res. 2005;57(2):169-76.
10. Grimes AC, Erwin KN, Stadt HA, Hunter GL, Gefroh HA, Tsai HJ, et al. PCB126 exposure disrupts zebrafish ventricular and branchial but not early neural crest development. Toxicol Sci. 2008;106(1):193-205.
11. van Gelder MM, van Rooij IA, Miller RK, Zielhuis GA, de Jong-van den Berg LT, Roeleveld N. Teratogenic mechanisms of medical drugs. Hum Reprod Update. 2010;16(4):378-94.
12. van Mierop LH. Diagnostic code for congenital heart disease. Pediatr Cardiol. 1984;5(4):331-62.
13. Van Mierop LH. Diagnostic code for congenital heart disease, supplement. Pediatr Cardiol. 1986;7(1):31-4.
14. Hagemo PS. BERTE--a database for children with congenital heart defects. Stud Health Technol Inform. 1994;14:98-101.
15. Botto LD, Lin AE, Riehle-Colarusso T, Malik S, Correa A, National Birth Defects Prevention S. Seeking causes: Classifying and evaluating congenital heart defects in etiologic studies. Birth Defects Res A Clin Mol Teratol. 2007;79(10):714-27.
16. Mason CA, Kirby RS, Sever LE, Langlois PH. Prevalence is the preferred measure of frequency of birth defects. Birth Defects Res A Clin Mol Teratol. 2005;73(10):690-2.
17. Hoffman JI, Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol. 2002;39(12):1890-900.
18. Botto LD, Lin AE, Riehle-Colarusso T, Malik S, Correa A. Seeking causes: Classifying and evaluating congenital heart defects in etiologic studies. Birth Defects Res A Clin Mol Teratol. 2007;79(10):714-27.

19. Øyen N, Poulsen G, Boyd HA, Wohlfahrt J, Jensen PK, Melbye M. National time trends in congenital heart defects, Denmark, 1977-2005. Am Heart J. 2009;157(3):467-73 e1.
20. van der Linde D, Konings EE, Slager MA, Witsenburg M, Helbing WA, Takkenberg JJ, et al. Birth prevalence of congenital heart disease worldwide: a systematic review and meta-analysis. J Am Coll Cardiol. 2011;58(21):2241-7.
21. Wren C, Richmond S, Donaldson L. Temporal variability in birth prevalence of cardiovascular malformations. Heart. 2000;83(4):414-9.
22. Ionescu-Ittu R, Marelli AJ, Mackie AS, Pilote L. Prevalence of severe congenital heart disease after folic acid fortification of grain products: time trend analysis in Quebec, Canada. BMJ. 2009;338:b1673.
23. Nora JJ. Multifactorial inheritance hypothesis for the etiology of congenital heart diseases. The genetic-environmental interaction. Circulation. 1968;38(3):604-17.
24. Jenkins KJ, Correa A, Feinstein JA, Botto L, Britt AE, Daniels SR, et al. Noninherited risk factors and congenital cardiovascular defects: current knowledge: a scientific statement from the American Heart Association Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. Circulation. 2007;115(23):2995-3014.
25. Nora JJ. Causes of congenital heart diseases: old and new modes, mechanisms, and models. Am Heart J. 1993;125(5 Pt 1):1409-19.
26. Richards AA, Garg V. Genetics of congenital heart disease. Curr Cardiol Rev. 2010;6(2):91-7.
27. Gelb BD. Genetic basis of congenital heart disease. Curr Opin Cardiol. 2004;19(2):110-5.
28. Øyen N, Poulsen G, Boyd HA, Wohlfahrt J, Jensen PK, Melbye M. Recurrence of congenital heart defects in families. Circulation. 2009;120(4):295-301.
29. Miller A, Riehle-Colarusso T, Siffel C, Frias JL, Correa A. Maternal age and prevalence of isolated congenital heart defects in an urban area of the United States. Am J Med Genet A. 2011;155A(9):2137-45.
30. Bahtiyar MO, Dulay AT, Weeks BP, Friedman AH, Copel JA. Prevalence of congenital heart defects in monochorionic/diamniotic twin gestations: a systematic literature review. J Ultrasound Med. 2007;26(11):1491-8.
31. Levy HL, Guldberg P, Guttler F, Hanley WB, Matalon R, Rouse BM, et al. Congenital heart disease in maternal phenylketonuria: report from the Maternal PKU Collaborative Study. Pediatr Res. 2001;49(5):636-42.
32. Dadvand P, Rankin J, Rushton S, Pless-Mulloli T. Ambient air pollution and congenital heart disease: a register-based study. Environ Res. 2011;111(3):435-41.
33. Alverson CJ, Strickland MJ, Gilboa SM, Correa A. Maternal smoking and congenital heart defects in the Baltimore-Washington Infant Study. Pediatrics. 2011;127(3):e647-53.
34. Stothard KJ, Tennant PW, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. JAMA. 2009;301(6):636-50.
35. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15(7):539-53.

36. Galtier F. Definition, epidemiology, risk factors. Diabetes Metab. 2010;36(6 Pt 2):628-51.
37. Folkehelseinstituttet. Årstabeller for Medisinsk fødselsregister 2010. 2012.
38. Midthjell K, Kruger O, Holmen J, Tverdal A, Claudi T, Bjorndal A, et al. Rapid changes in the prevalence of obesity and known diabetes in an adult Norwegian population. The Nord-Trondelag Health Surveys: 1984-1986 and 1995-1997. Diabetes Care. 1999;22(11):1813-20.
39. Tieu J, Middleton P, Crowther CA. Preconception care for diabetic women for improving maternal and infant health. Cochrane Database Syst Rev. 2010(12):CD007776.
40. Kitzmiller JL, Wallerstein R, Correa A, Kwan S. Preconception care for women with diabetes and prevention of major congenital malformations. Birth Defects Res A Clin Mol Teratol. 2010;88(10):791-803.
41. Bell R, Bailey K, Cresswell T, Hawthorne G, Critchley J, Lewis-Barned N. Trends in prevalence and outcomes of pregnancy in women with pre-existing type I and type II diabetes. BJOG. 2008;115(4):445-52.
42. American Diabetes A. Gestational diabetes mellitus. Diabetes Care. 2004;27 Suppl 1:S88-90.
43. Buchanan TA, Xiang A, Kjos SL, Watanabe R. What is gestational diabetes? Diabetes Care. 2007;30 Suppl 2:S105-11.
44. Barbour LA, McCurdy CE, Hernandez TL, Kirwan JP, Catalano PM, Friedman JE. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. Diabetes Care. 2007;30 Suppl 2:S112-9.
45. Petry CJ. Gestational diabetes: risk factors and recent advances in its genetics and treatment. Br J Nutr. 2010;104(6):775-87.
46. Hunt KJ, Schuller KL. The increasing prevalence of diabetes in pregnancy. Obstet Gynecol Clin North Am. 2007;34(2):173-99, vii.
47. Engeland A, Bjorge T, Daltveit AK, Skurtveit S, Vangen S, Vollset SE, et al. Risk of diabetes after gestational diabetes and preeclampsia. A registry-based study of 230,000 women in Norway. Eur J Epidemiol. 2011;26(2):157-63.
48. Klovning A, Backe B, Roland B. Nye nasjonale retningslinjer for gravide (pdf). Oslo: Sosial-og helsedirekoratet. 2005.
49. Corrigan N, Brazil DP, McAuliffe F. Fetal cardiac effects of maternal hyperglycemia during pregnancy. Birth Defects Res A Clin Mol Teratol. 2009;85(6):523-30.
50. Eidem I, Stene LC, Henriksen T, Hanssen KF, Vangen S, Vollset SE, et al. Congenital anomalies in newborns of women with type 1 diabetes: nationwide population-based study in Norway, 1999-2004. Acta Obstet Gynecol Scand. 2010;89(11):1403-11.
51. Macintosh MC, Fleming KM, Bailey JA, Doyle P, Modder J, Acolet D, et al. Perinatal mortality and congenital anomalies in babies of women with type 1 or type 2 diabetes in England, Wales, and Northern Ireland: population based study. BMJ. 2006;333(7560):177.
52. Lisowski LA, Verheijen PM, Copel JA, Kleinman CS, Wassink S, Visser GH, et al. Congenital heart disease in pregnancies complicated by maternal diabetes mellitus. An international clinical collaboration, literature review, and meta-analysis. Herz. 2010;35(1):19-26.

53. Schaefer-Graf UM, Buchanan TA, Xiang A, Songster G, Montoro M, Kjos SL. Patterns of congenital anomalies and relationship to initial maternal fasting glucose levels in pregnancies complicated by type 2 and gestational diabetes. Am J Obstet Gynecol. 2000;182(2):313-20.
54. Walkinshaw SA. Pregnancy in women with pre-existing diabetes: management issues. Semin Fetal Neonatal Med. 2005;10(4):307-15.
55. Baumann MU, Deborde S, Illsley NP. Placental glucose transfer and fetal growth. Endocrine. 2002;19(1):13-22.
56. Oakley NW, Beard RW, Turner RC. Effect of sustained maternal hyperglycaemia on the fetus in normal and diabetic pregnancies. Br Med J. 1972;1(5798):466-9.
57. Eriksson UJ, Cederberg J, Wentzel P. Congenital malformations in offspring of diabetic mothers--animal and human studies. Rev Endocr Metab Disord. 2003;4(1):79-93.
58. Gilbert-Barness E. Teratogenic causes of malformations. Ann Clin Lab Sci. 2010;40(2):99-114.
59. Morgan SC, Relaix F, Sandell LL, Loeken MR. Oxidative stress during diabetic pregnancy disrupts cardiac neural crest migration and causes outflow tract defects. Birth Defects Res A Clin Mol Teratol. 2008;82(6):453-63.
60. Haram K, Bergsjo P, Pirhonen J. [Suspected large fetus in the last period of pregnancy--a difficult problem]. Tidsskr Nor Laegeforen. 2001;121(11):1369-73.
61. Jolly MC, Sebire NJ, Harris JP, Regan L, Robinson S. Risk factors for macrosomia and its clinical consequences: a study of 350,311 pregnancies. Eur J Obstet Gynecol Reprod Biol. 2003;111(1):9-14.
62. Ehrenberg HM, Mercer BM, Catalano PM. The influence of obesity and diabetes on the prevalence of macrosomia. Am J Obstet Gynecol. 2004;191(3):964-8.
63. Hay WW, Jr. Placental-fetal glucose exchange and fetal glucose metabolism. Trans Am Clin Climatol Assoc. 2006;117:321-39; discussion 39-40.
64. Leipold H, Worda C, Gruber CJ, Kautzky-Willer A, Husslein PW, Bancher-Todesca D. Large-for-gestational-age newborns in women with insulin-treated gestational diabetes under strict metabolic control. Wien Klin Wochenschr. 2005;117(15-16):521-5.
65. Ahlsson F, Diderholm B, Jonsson B, Norden-Lindberg S, Olsson R, Ewald U, et al. Insulin resistance, a link between maternal overweight and fetal macrosomia in nondiabetic pregnancies. Horm Res Paediatr. 2010;74(4):267-74.
66. Voldner N, Qvigstad E, Froslie KF, Godang K, Henriksen T, Bollerslev J. Increased risk of macrosomia among overweight women with high gestational rise in fasting glucose. J Matern Fetal Neonatal Med. 2010;23(1):74-81.
67. Van Assche FA. Fetal growth and development. Verh K Acad Geneeskd Belg. 1998;60(1):3-11; discussion -2.
68. Van Assche FA, Holemans K, Aerts L. Long-term consequences for offspring of diabetes during pregnancy. Br Med Bull. 2001;60:173-82.
69. Stover PJ. Physiology of folate and vitamin B12 in health and disease. Nutr Rev. 2004;62(6 Pt 2):S3-12; discussion S3.
70. Winkels RM, Brouwer IA, Siebelink E, Katan MB, Verhoef P. Bioavailability of food folates is 80% of that of folic acid. Am J Clin Nutr. 2007;85(2):465-73.
71. Antony AC. In utero physiology: role of folic acid in nutrient delivery and fetal development. Am J Clin Nutr. 2007;85(2):598S-603S.

72. Rosenquist TH, Chaudoin T, Finnell RH, Bennett GD. High-affinity folate receptor in cardiac neural crest migration: a gene knockdown model using siRNA. Dev Dyn. 2010;239(4):1136-44.
73. Henderson GI, Perez T, Schenker S, Mackins J, Antony AC. Maternal-to-fetal transfer of 5-methyltetrahydrofolate by the perfused human placental cotyledon: evidence for a concentrative role by placental folate receptors in fetal folate delivery. J Lab Clin Med. 1995;126(2):184-203.
74. Milunsky A, Jick H, Jick SS, Bruell CL, MacLaughlin DS, Rothman KJ, et al. Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. JAMA. 1989;262(20):2847-52.
75. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med. 1992;327(26):1832-5.
76. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. Lancet. 1991;338(8760):131-7.
77. Werler MM, Shapiro S, Mitchell AA. Periconceptional folic acid exposure and risk of occurrent neural tube defects. JAMA. 1993;269(10):1257-61.
78. Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. N Engl J Med. 1999;341(20):1485-90.
79. Botto LD, Mulinare J, Erickson JD. Do multivitamin or folic acid supplements reduce the risk for congenital heart defects? Evidence and gaps. Am J Med Genet A. 2003;121A(2):95-101.
80. Shaw GM, O'Malley CD, Wasserman CR, Tolarova MM, Lammer EJ. Maternal periconceptional use of multivitamins and reduced risk for conotruncal heart defects and limb deficiencies among offspring. Am J Med Genet. 1995;59(4):536-45.
81. Czeizel AE. Periconceptional folic acid containing multivitamin supplementation. Eur J Obstet Gynecol Reprod Biol. 1998;78(2):151-61.
82. van Beynum IM, Kapusta L, Bakker MK, den Heijer M, Blom HJ, de Walle HE. Protective effect of periconceptional folic acid supplements on the risk of congenital heart defects: a registry-based case-control study in the northern Netherlands. Eur Heart J. 2010;31(4):464-71.
83. Godwin KA, Sibbald B, Bedard T, Kuzeljevic B, Lowry RB, Arbour L. Changes in frequencies of select congenital anomalies since the onset of folic acid fortification in a Canadian birth defect registry. Can J Public Health. 2008;99(4):271-5.
84. Tamura T, Picciano MF. Folate and human reproduction. Am J Clin Nutr. 2006;83(5):993-1016.
85. Gardiner PM, Nelson L, Shellhaas CS, Dunlop AL, Long R, Andrist S, et al. The clinical content of preconception care: nutrition and dietary supplements. Am J Obstet Gynecol. 2008;199(6 Suppl 2):S345-56.
86. Gardiner HM, Fouron JC. Folic acid fortification and congenital heart disease. BMJ. 2009;338:b1144.
87. Mattilsynet. Innvilgede søknader om tlllatelse til å tilsette vitaminer og/eller mineraler til næringsmidler. 2015.

88. ernæringsråd S. Anbefalinger og virkemidler for økt folatinntak blant kvinner i fertil alder. . 1998.
89. Daltveit AK, Vollset SE, Lande B, Oien H. Changes in knowledge and attitudes of folate, and use of dietary supplements among women of reproductive age in Norway 1998-2000. Scand J Public Health. 2004;32(4):264-71.
90. Kim JH, Scialli AR. Thalidomide: the tragedy of birth defects and the effective treatment of disease. Toxicol Sci. 2011;122(1):1-6.
91. Melve KK, Lie RT, Skjaerven R, Van Der Hagen CB, Gradek GA, Jonsrud C, et al. Registration of Down syndrome in the Medical Birth Registry of Norway: validity and time trends. Acta Obstet Gynecol Scand. 2008;87(8):824-30.
92. Irgens LM. The Medical Birth Registry of Norway; a source for epidemiological and clinical research. Scand J Rheumatol Suppl. 1998;107:105-8.
93. Folkehelseinstituttet. Annual Report 2001-2002, Medical Birth Registry of Norway [Available from: http://www.fhi.no/dav/f1065301e2.pdf.
94. CVDNOR Cardiovascular Disease in Norway [Available from: http://www.cvdnor.no.
95. Sulo G IJ, Vollset SE, Nygård O, Øyen N, Tell GS. Cardiovascular disease and diabetes mellitus in Norway during 1994-2009 CVDNOR – a nationwide research project. Norwegian journal of epidemiology. 2013;23(1):101-7.
96. Pedersen AG, Ellingsen CL. Data quality in the Causes of Death Registry. Tidsskr Nor Laegeforen. 2015;135(8):768-70.
97. Statistics Norway. [Available from: http://www.ssb.no/en/forside.
98. Rodriguez RJ, Riggs TW. Physiologic peripheral pulmonic stenosis in infancy. Am J Cardiol. 1990;66(20):1478-81.
99. Ulster ECRUo. EUROCAT special report: congenital heart defects in Europe, 2000-2005. 2009.
100. Skjaerven R, Gjessing HK, Bakketeig LS. Birthweight by gestational age in Norway. Acta Obstet Gynecol Scand. 2000;79(6):440-9.
101. Hediger ML, Overpeck MD, Kuczmarski RJ, McGlynn A, Maurer KR, Davis WW. Muscularity and fatness of infants and young children born small- or large-for-gestational-age. Pediatrics. 1998;102(5):E60.
102. Nilsen RM, Vollset SE, Rasmussen SA, Ueland PM, Daltveit AK. Folic acid and multivitamin supplement use and risk of placental abruption: a population-based registry study. Am J Epidemiol. 2008;167(7):867-74.
103. Wacholder S. Binomial regression in GLIM: estimating risk ratios and risk differences. Am J Epidemiol. 1986;123(1):174-84.
104. Kim HJ, Fay MP, Feuer EJ, Midthune DN. Permutation tests for joinpoint regression with applications to cancer rates. Stat Med. 2000;19(3):335-51.
105. Bjornard K, Riehle-Colarusso T, Gilboa SM, Correa A. Patterns in the prevalence of congenital heart defects, metropolitan Atlanta, 1978 to 2005. Birth Defects Res A Clin Mol Teratol. 2013;97(2):87-94.
106. Ho PM, Peterson PN, Masoudi FA. Evaluating the evidence: is there a rigid hierarchy? Circulation. 2008;118(16):1675-84.
107. Loane M, Dolk H, Kelly A, Teljeur C, Greenlees R, Densem J, et al. Paper 4: EUROCAT statistical monitoring: identification and investigation of ten year trends of

congenital anomalies in Europe. Birth Defects Res A Clin Mol Teratol. 2011;91 Suppl 1:S31-43.
108. Stene LC, Eidem I, Vangen S, Joner G, Irgens L, Moe N. The validity of the diabetes mellitus diagnosis in the Medical Birth Registry of Norway. Norsk Epidemiologi. 2007;17(2):165-74.
109. Aamodt G, Stene LC, Njolstad PR, Sovik O, Joner G, Norwegian Childhood Diabetes Study G. Spatiotemporal trends and age-period-cohort modeling of the incidence of type 1 diabetes among children aged <15 years in Norway 1973-1982 and 1989-2003. Diabetes Care. 2007;30(4):884-9.
110. Vollset SE, Gjessing HK, Tandberg A, Ronning T, Irgens LM, Baste V, et al. Folate supplementation and twin pregnancies. Epidemiology. 2005;16(2):201-5.
111. Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, et al. Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. JAMA. 2013;309(6):570-7.
112. Delgado-Rodriguez M, Llorca J. Bias. J Epidemiol Community Health. 2004;58(8):635-41.
113. Rothman KJ. Epidemiology : an introduction. New York, N.Y.: Oxford University Press; 2002. viii, 223 p. p.
114. Reece EA, Homko CJ. Diabetes-related complications of pregnancy. J Natl Med Assoc. 1993;85(7):537-45.
115. Bird Y, Lemstra M, Rogers M, Moraros J. The relationship between socioeconomic status/income and prevalence of diabetes and associated conditions: A cross-sectional population-based study in Saskatchewan, Canada. Int J Equity Health. 2015;14:93.
116. Langlois PH, Scheuerle A, Horel SA, Carozza SE. Urban versus rural residence and occurrence of septal heart defects in Texas. Birth Defects Res A Clin Mol Teratol. 2009;85(9):764-72.
117. Ben-Haroush A, Yogev Y, Hod M. Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. Diabet Med. 2004;21(2):103-13.
118. Veiby G, Daltveit AK, Engelsen BA, Gilhus NE. Pregnancy, delivery, and outcome for the child in maternal epilepsy. Epilepsia. 2009;50(9):2130-9.
119. Kuciene R, Dulskiene V. Maternal socioeconomic and lifestyle factors during pregnancy and the risk of congenital heart defects. Medicina (Kaunas). 2009;45(11):904-9.
120. Nilsen RM, Vollset SE, Gjessing HK, Magnus P, Meltzer HM, Haugen M, et al. Patterns and predictors of folic acid supplement use among pregnant women: the Norwegian Mother and Child Cohort Study. Am J Clin Nutr. 2006;84(5):1134-41.
121. Khoshnood B, Loane M, Garne E, Addor MC, Arriola L, Bakker M, et al. Recent Decrease in the Prevalence of Congenital Heart Defects in Europe. J Pediatr. 2012.
122. Allen VM, Armson BA, Wilson RD, Allen VM, Blight C, Gagnon A, et al. Teratogenicity associated with pre-existing and gestational diabetes. J Obstet Gynaecol Can. 2007;29(11):927-44.
123. Moazzen H, Lu X, Liu M, Feng Q. Pregestational Diabetes Induces Fetal Coronary Artery Malformation via Reactive Oxygen Species Signaling. Diabetes. 2014.
124. Correa A, Gilboa SM, Besser LM, Botto LD, Moore CA, Hobbs CA, et al. Diabetes mellitus and birth defects. Am J Obstet Gynecol. 2008;199(3):237 e1-9.

125. Feig DS, Hwee J, Shah BR, Booth GL, Bierman AS, Lipscombe LL. Trends in incidence of diabetes in pregnancy and serious perinatal outcomes: a large, population-based study in Ontario, Canada, 1996-2010. Diabetes Care. 2014;37(6):1590-6.
126. Feig DS, Briggs GG, Koren G. Oral antidiabetic agents in pregnancy and lactation: a paradigm shift? Ann Pharmacother. 2007;41(7):1174-80.
127. Mathiesen ER, Kinsley B, Amiel SA, Heller S, McCance D, Duran S, et al. Maternal glycemic control and hypoglycemia in type 1 diabetic pregnancy: a randomized trial of insulin aspart versus human insulin in 322 pregnant women. Diabetes Care. 2007;30(4):771-6.
128. Bokariya P, Kothari R, Gujar VK, Shende MR. Teratogenic effects of insulin: an experimental study on developing chick embryo. Indian J Pharmacol. 2015;47(2):212-4.
129. Kanda E, Matsuda Y, Makino Y, Matsui H. Risk factors associated with altered fetal growth in patients with pregestational diabetes mellitus. J Matern Fetal Neonatal Med. 2012;25(8):1390-4.
130. Ellerbe CN, Gebregziabher M, Korte JE, Mauldin J, Hunt KJ. Quantifying the impact of gestational diabetes mellitus, maternal weight and race on birthweight via quantile regression. PLoS One. 2013;8(6):e65017.
131. Parker SE, Werler MM, Shaw GM, Anderka M, Yazdy MM, National Birth Defects Prevention S. Dietary glycemic index and the risk of birth defects. Am J Epidemiol. 2012;176(12):1110-20.
132. Leirgul E, Fomina T, Brodwall K, Greve G, Holmstrom H, Vollset SE, et al. Birth prevalence of congenital heart defects in Norway 1994-2009-A nationwide study. Am Heart J. 2014;168(6):956-64.
133. Bedard T, Lowry RB, Sibbald B, Harder JR, Trevenen C, Horobec V, et al. Folic acid fortification and the birth prevalence of congenital heart defect cases in Alberta, Canada. Birth Defects Res A Clin Mol Teratol. 2013;97(8):564-70.
134. Csaky-Szunyogh M, Vereczkey A, Kosa Z, Gerencser B, Czeizel AE. Risk and protective factors in the origin of conotruncal defects of heart--a population-based case-control study. Am J Med Genet A. 2013;161A(10):2444-52.
135. Sengpiel V, Bacelis J, Myhre R, Myking S, Devold Pay A, Haugen M, et al. Folic acid supplementation, dietary folate intake during pregnancy and risk for spontaneous preterm delivery: a prospective observational cohort study. BMC Pregnancy Childbirth. 2014;14(1):375.
136. Li X, Li S, Mu D, Liu Z, Li Y, Lin Y, et al. The association between periconceptional folic acid supplementation and congenital heart defects: a case-control study in China. Prev Med. 2013;56(6):385-9.
137. Roth C, Bjorke-Monsen AL, Reichborn-Kjennerud T, Nilsen RM, Smith GD, Stoltenberg C, et al. Use of folic acid supplements in early pregnancy in relation to maternal plasma levels in week 18 of pregnancy. Mol Nutr Food Res. 2013;57(4):653-60.
138. Kim KC, Friso S, Choi SW. DNA methylation, an epigenetic mechanism connecting folate to healthy embryonic development and aging. J Nutr Biochem. 2009;20(12):917-26.
139. Mikael LG, Deng L, Paul L, Selhub J, Rozen R. Moderately high intake of folic acid has a negative impact on mouse embryonic development. Birth Defects Res A Clin Mol Teratol. 2013;97(1):47-52.

140. Pickell L, Brown K, Li D, Wang XL, Deng L, Wu Q, et al. High intake of folic acid disrupts embryonic development in mice. Birth Defects Res A Clin Mol Teratol. 2011;91(1):8-19.
141. Crider KS, Bailey LB, Berry RJ. Folic acid food fortification-its history, effect, concerns, and future directions. Nutrients. 2011;3(3):370-84.
142. Mortensen JH, Oyen N, Fomina T, Melbye M, Tretli S, Vollset SE, et al. Supplemental folic acid in pregnancy and maternal cancer risk. Cancer Epidemiol. 2015;39(6):805-11.
143. Mortensen JH, Oyen N, Fomina T, Melbye M, Tretli S, Vollset SE, et al. Supplemental folic acid in pregnancy and childhood cancer risk. Br J Cancer. 2016;114(1):71-5.
144. EUROCAT european surveillance of congenital anomalies [Available from: http://www.eurocat-network.eu.
145. Priest JR, Yang W, Reaven G, Knowles JW, Shaw GM. Maternal Midpregnancy Glucose Levels and Risk of Congenital Heart Disease in Offspring. JAMA Pediatr. 2015;169(12):1112-6.
146. Brodwall K, Leirgul E, Greve G, Vollset SE, Holmstrom H, Tell GS, et al. Possible Common Aetiology behind Maternal Preeclampsia and Congenital Heart Defects in the Child: a Cardiovascular Diseases in Norway Project Study. Paediatr Perinat Epidemiol. 2015.
147. Auger N, Fraser WD, Healy-Profitos J, Arbour L. Association Between Preeclampsia and Congenital Heart Defects. JAMA. 2015;314(15):1588-98.
148. Avogaro A, Albiero M, Menegazzo L, de Kreutzenberg S, Fadini GP. Endothelial dysfunction in diabetes: the role of reparatory mechanisms. Diabetes Care. 2011;34 Suppl 2:S285-90.
149. American Diabetes A. Preconception care of women with diabetes. Diabetes Care. 2004;27 Suppl 1:S76-8.

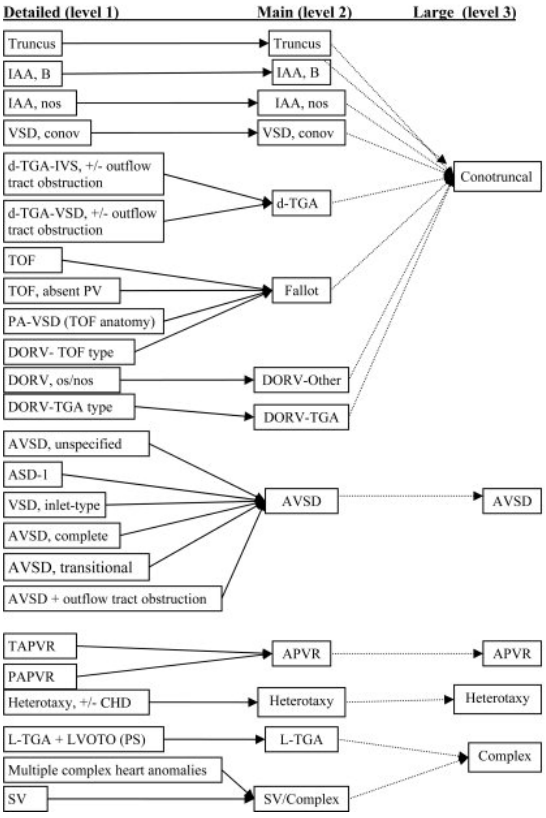
Appendices

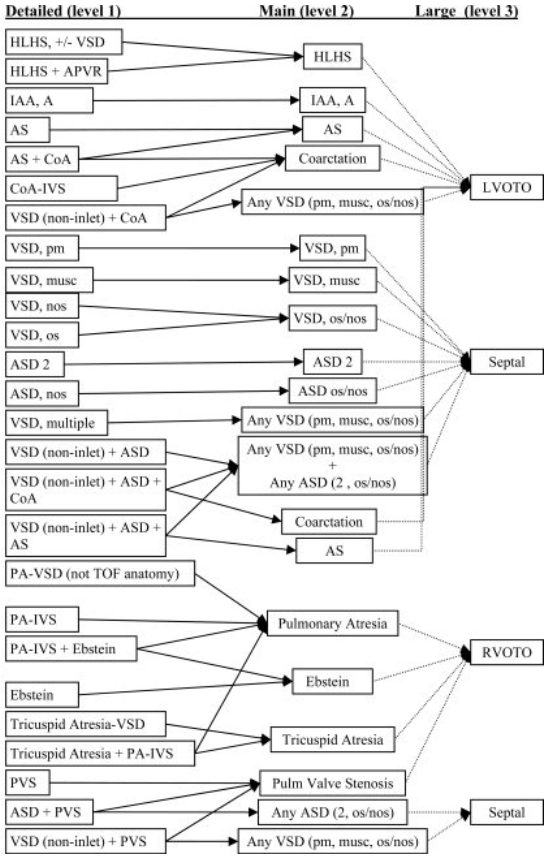
1. The classification by Botto et al

2. The Medical Birth Registry of Norway birth notification form until 1998

3. The revised MBRN birth notification form, December 1998

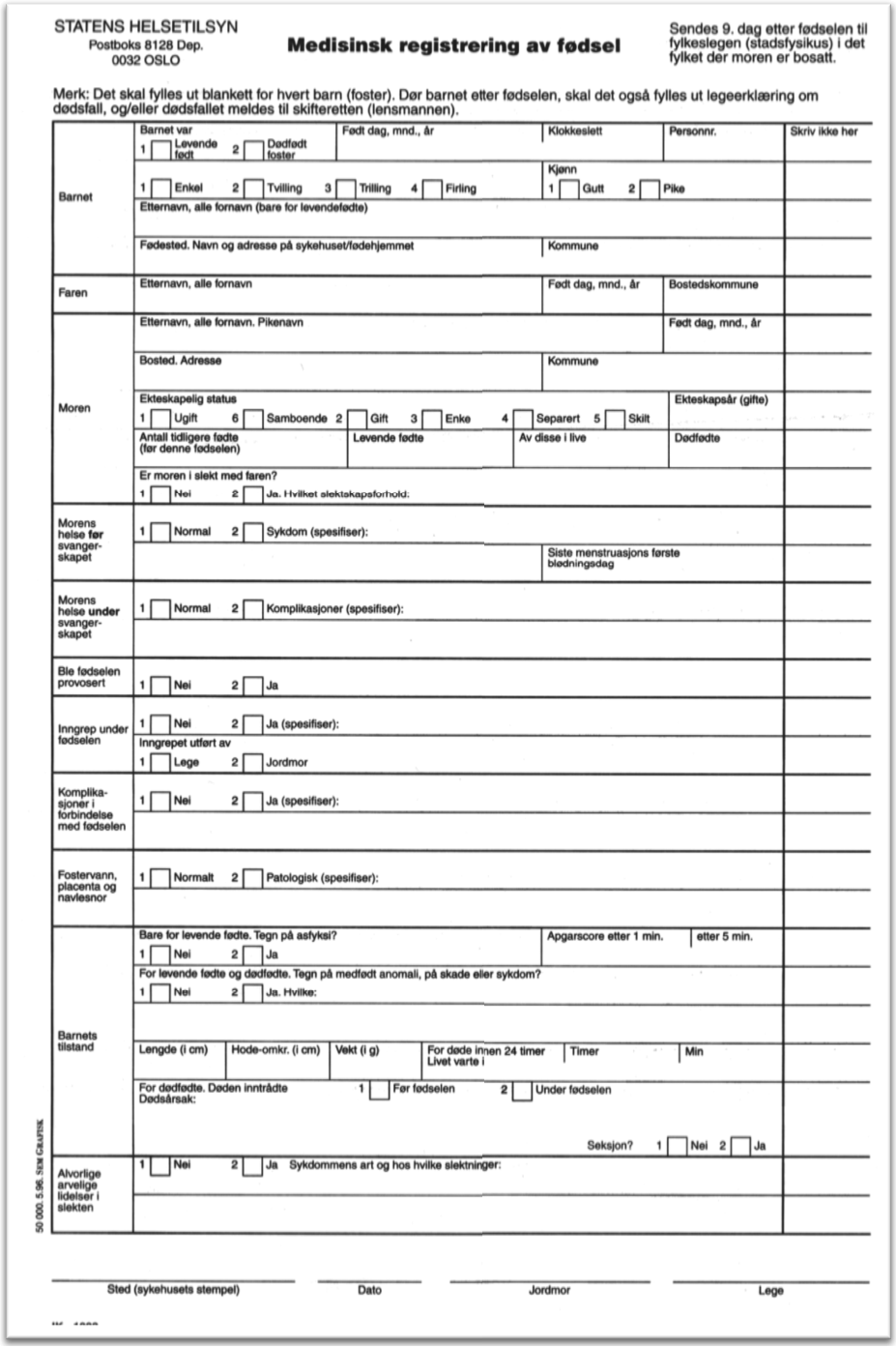
The classification system by Botto et al





Reprinted with permission from John Wiley and Sons

The MBRN birth notification form 1967-1998



STATENS HELSETILSYN
Postboks 8128 Dep.
0032 OSLO

Medisinsk registrering av fødsel

Sendes 9. dag etter fødselen til fylkeslegen (stadsfysikus) i det fylket der moren er bosatt.

Merk: Det skal fylles ut blankett for hvert barn (foster). Dør barnet etter fødselen, skal det også fylles ut legeerklæring om dødsfall, og/eller dødsfallet meldes til skifteretten (lensmannen).

Barnet
Barnet var 1 ☐ Levende født 2 ☐ Dødfødt foster | Født dag, mnd., år | Klokkeslett | Personnr. | Skriv ikke her
1 ☐ Enkel 2 ☐ Tvilling 3 ☐ Trilling 4 ☐ Firling | Kjønn 1 ☐ Gutt 2 ☐ Pike
Etternavn, alle fornavn (bare for levendefødte)
Fødested. Navn og adresse på sykehuset/fødehjemmet | Kommune

Faren
Etternavn, alle fornavn | Født dag, mnd., år | Bostedskommune

Moren
Etternavn, alle fornavn. Pikenavn | Født dag, mnd., år
Bosted. Adresse | Kommune
Ekteskapelig status 1 ☐ Ugift 6 ☐ Samboende 2 ☐ Gift 3 ☐ Enke 4 ☐ Separert 5 ☐ Skilt | Ekteskapsår (gifte)
Antall tidligere fødte (før denne fødselen) | Levende fødte | Av disse i live | Dødfødte
Er moren i slekt med faren? 1 ☐ Nei 2 ☐ Ja. Hvilket slektskapsforhold:

Morens helse før svangerskapet
1 ☐ Normal 2 ☐ Sykdom (spesifiser):
Siste menstruasjons første blødningsdag

Morens helse under svangerskapet
1 ☐ Normal 2 ☐ Komplikasjoner (spesifiser):

Ble fødselen provosert
1 ☐ Nei 2 ☐ Ja

Inngrep under fødselen
1 ☐ Nei 2 ☐ Ja (spesifiser):
Inngrepet utført av 1 ☐ Lege 2 ☐ Jordmor

Komplikasjoner i forbindelse med fødselen
1 ☐ Nei 2 ☐ Ja (spesifiser):

Fostervann, placenta og navlesnor
1 ☐ Normalt 2 ☐ Patologisk (spesifiser):

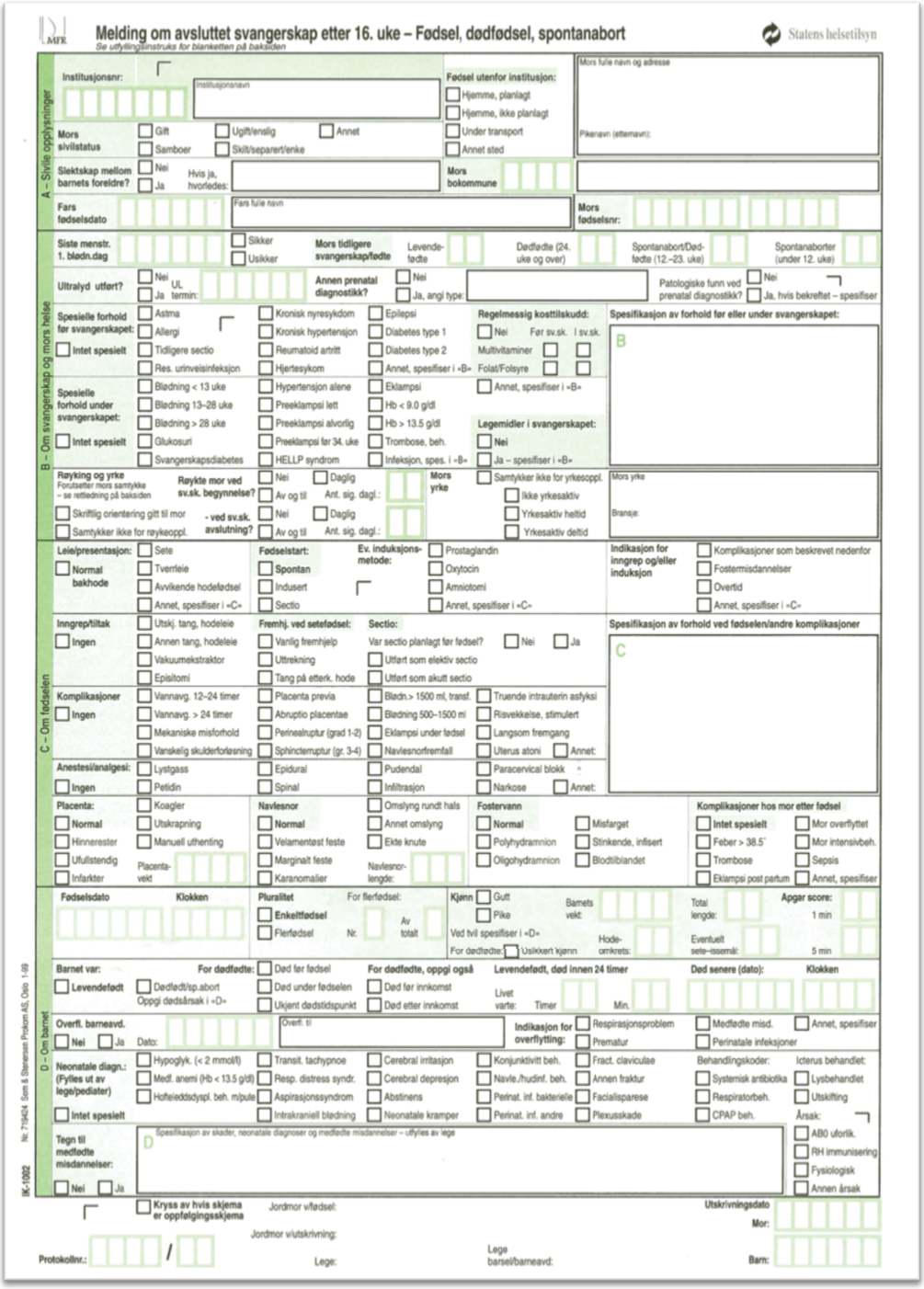
Barnets tilstand
Bare for levende fødte. Tegn på asfyksi? 1 ☐ Nei 2 ☐ Ja | Apgarscore etter 1 min. | etter 5 min.
For levende fødte og dødfødte. Tegn på medfødt anomali, på skade eller sykdom? 1 ☐ Nei 2 ☐ Ja. Hvilke:
Lengde (i cm) | Hode-omkr. (i cm) | Vekt (i g) | For døde innen 24 timer Livet varte i | Timer | Min
For dødfødte. Døden inntrådte 1 ☐ Før fødselen 2 ☐ Under fødselen
Dødsårsak:
Seksjon? 1 ☐ Nei 2 ☐ Ja

Alvorlige arvelige lidelser i slekten
1 ☐ Nei 2 ☐ Ja Sykdommens art og hos hvilke slektninger:

Sted (sykehusets stempel) | Dato | Jordmor | Lege

50 000. 5.86. Stat Grafisk

The revised MBRN birth notification form, 1998



Melding om avsluttet svangerskap etter 16. uke – Fødsel, dødfødsel, spontanabort

Se utfyllingsinstruks for blanketten på baksiden

Statens helsetilsyn

A – Sivile opplysninger

Institusjonsnr: | Institusjonsnavn | Fødsel utenfor institusjon: Hjemme, planlagt; Hjemme, ikke planlagt; Under transport; Annet sted | Mors fulle navn og adresse | Pikenavn (etternavn):

Mors sivilstatus: Gift; Ugift/enslig; Annet; Samboer; Skilt/separert/enke

Slektskap mellom barnets foreldre? Nei; Ja; Hvis ja, hvorledes:

Mors bokommune

Fars fødselsdato | Fars fulle navn | Mors fødselsnr:

B – Om svangerskap og mors helse

Siste menstr. 1. blødn.dag | Sikker; Usikker | Mors tidligere svangerskap/fødte: Levendefødte; Dødfødte (24. uke og over); Spontanabort/Dødfødte (12.–23. uke); Spontanaborter (under 12. uke)

Ultralyd utført? Nei; Ja; UL termin: | Annen prenatal diagnostikk? Nei; Ja, angi type: | Patologiske funn ved prenatal diagnostikk? Nei; Ja, hvis bekreftet – spesifiser

Spesielle forhold før svangerskapet: Intet spesielt; Astma; Allergi; Tidligere sectio; Res. urinveisinfeksjon; Kronisk nyresykdom; Kronisk hypertensjon; Reumatoid artritt; Hjertesykom; Epilepsi; Diabetes type 1; Diabetes type 2; Annet, spesifiser i «B»

Regelmessig kosttilskudd: Nei; Før sv.sk.; I sv.sk.; Multivitaminer; Folat/Folsyre; Annet, spesifiser i «B»

Spesifikasjon av forhold før eller under svangerskapet: B

Spesielle forhold under svangerskapet: Intet spesielt; Blødning < 13 uke; Blødning 13–28 uke; Blødning > 28 uke; Glukosuri; Svangerskapsdiabetes; Hypertensjon alene; Preeklampsi lett; Preeklampsi alvorlig; Preeklampsi før 34. uke; HELLP syndrom; Eklampsi; Hb < 9.0 g/dl; Hb > 13.5 g/dl; Trombose, beh.; Infeksjon, spes. i «B»

Legemidler i svangerskapet: Nei; Ja – spesifiser i «B»

Røyking og yrke (Forutsetter mors samtykke – se rettledning på baksiden): Skriftlig orientering gitt til mor; Samtykker ikke for røykeoppl.

Røykte mor ved sv.sk. begynnelse? Nei; Daglig; Av og til; Ant. sig. dagl.:

– ved sv.sk. avslutning? Nei; Daglig; Av og til; Ant. sig. dagl.:

Mors yrke: Samtykker ikke for yrkesoppl.; Ikke yrkesaktiv; Yrkesaktiv heltid; Yrkesaktiv deltid | Mors yrke | Bransje:

C – Om fødselen

Leie/presentasjon: Normal bakhode; Sete; Tverrleie; Avvikende hodefødsel; Annet, spesifiser i «C»

Fødselstart: Spontan; Indusert; Sectio

Ev. induksjonsmetode: Prostaglandin; Oxytocin; Amniotomi; Annet, spesifiser i «C»

Indikasjon for inngrep og/eller induksjon: Komplikasjoner som beskrevet nedenfor; Fostermisdannelser; Overtid; Annet, spesifiser i «C»

Inngrep/tiltak: Ingen; Utskj. tang, hodeleie; Annen tang, hodeleie; Vakuumekstraktor; Episiotomi

Fremhj. ved setefødsel: Vanlig fremhjelp; Uttrekning; Tang på etterk. hode

Sectio: Var sectio planlagt før fødsel? Nei; Ja; Utført som elektiv sectio; Utført som akutt sectio

Spesifikasjon av forhold ved fødselen/andre komplikasjoner: C

Komplikasjoner: Ingen; Vannavg. 12–24 timer; Vannavg. > 24 timer; Mekaniske misforhold; Vanskelig skulderforløsning; Placenta previa; Abruptio placentae; Perinealruptur (grad 1-2); Sphincterruptur (gr. 3-4); Blødn.> 1500 ml, transf.; Blødning 500–1500 ml; Eklampsi under fødsel; Navlesnorfremfall; Truende intrauterin asfyksi; Risvekkelse, stimulert; Langsom fremgang; Uterus atoni; Annet:

Anestesi/analgesi: Ingen; Lystgass; Petidin; Epidural; Spinal; Pudendal; Infiltrasjon; Paracervical blokk; Narkose; Annet:

Placenta: Normal; Hinnerester; Ufullstendig; Infarkter; Koagler; Utskrapning; Manuell uthenting; Placenta-vekt

Navlesnor: Normal; Velamentøst feste; Marginalt feste; Karanomalier; Omslyng rundt hals; Annet omslyng; Ekte knute; Navlesnor-lengde:

Fostervann: Normal; Polyhydramnion; Oligohydramnion; Misfarget; Stinkende, infisert; Blodtiblandet

Komplikasjoner hos mor etter fødsel: Intet spesielt; Feber > 38,5°; Trombose; Eklampsi post partum; Mor overflyttet; Mor intensivbeh.; Sepsis; Annet, spesifiser

D – Om barnet

Fødselsdato | Klokken | Pluralitet: Enkeltfødsel; Flerfødsel; For flerfødsel: Nr.; Av totalt | Kjønn: Gutt; Pike; Ved tvil spesifiser i «D»; For dødfødte: Uslikkert kjønn | Barnets vekt: | Hode-omkrets: | Total lengde: | Eventuelt sete–issemål: | Apgar score: 1 min; 5 min

Barnet var: Levendefødt; Dødfødt/sp.abort Oppgi dødsårsak i «D»

For dødfødte: Død før fødsel; Død under fødselen; Ukjent dødstidspunkt

For dødfødte, oppgi også: Død før innkomst; Død etter innkomst

Levendefødt, død innen 24 timer: Livet varte: Timer; Min.

Død senere (dato): | Klokken

Overfl. barneavd. Nei; Ja | Dato: | Overfl. til | Indikasjon for overflytting: Respirasjonsproblem; Prematur; Medfødte misd.; Perinatale infeksjoner; Annet, spesifiser

Neonatale diagn.: (Fylles ut av lege/pediater) Intet spesielt; Hypoglyk. (< 2 mmol/l); Medf. anemi (Hb < 13.5 g/dl); Hofteleddsdyspl. beh. m/pute; Transit. tachypnoe; Resp. distress syndr.; Aspirasjonssyndrom; Intrakraniell blødning; Cerebral irritasjon; Cerebral depresjon; Abstinens; Neonatale kramper; Konjunktivitt beh.; Navle./hudinf. beh.; Perinat. inf. bakterielle; Perinat. inf. andre; Fract. claviculae; Annen fraktur; Facialisparese; Plexusskade

Behandlingskoder: Systemisk antibiotika; Respiratorbeh.; CPAP beh.

Icterus behandlet: Lysbehandlet; Utskifting

Årsak: AB0 uforlik.; RH immunisering; Fysiologisk; Annen årsak

Tegn til medfødte misdannelser: Nei; Ja | D – Spesifikasjon av skader, neonatale diagnoser og medfødte misdannelser – utfylles av lege

Kryss av hvis skjema er oppfølgingsskjema

Jordmor v/fødsel: | Jordmor v/utskrivning: | Lege: | Lege barsel/barneavd:

Utskrivningsdato Mor: | Barn:

Protokollnr.:

MBR | Nr. 719424 Sem & Stenersen Prokom AS, Oslo 1-99 | IK-1002

I

Birth prevalence of congenital heart defects in Norway 1994-2009—A nationwide study

Elisabeth Leirgul, MD,[a,b] Tatiana Fomina, PhD,[a] Kristoffer Brodwall, MD,[a,c] Gottfried Greve, MD, PhD,[b,d] Henrik Holmstrøm, MD, PhD,[e] Stein Emil Vollset, MD, DrPH,[a,f] Grethe S. Tell, MPH, PhD,[a,f] and Nina Øyen, MD, MPH, DrMed[a,g] *Bergen, and Oslo, Norway*

Background The reasons for decreasing birth prevalence of congenital heart defects (CHDs) in several European countries and Canada are not fully understood. We present CHD prevalence among live births, stillbirths, and terminated pregnancies in an entire nation over a period of 16 years.

Methods Information on all births in the Medical Birth Registry of Norway, 1994-2009, was updated with information on CHD from the hospitals' Patient Administrative Systems, the National Hospital's clinical database for children with heart disease, and the Cause of Death Registry. Individuals with heart defects were assigned specific cardiac phenotypes.

Results Among 954,413 births, 13,081 received a diagnosis of CHD (137.1 per 10,000 births, 133.2 per 10,000 live births). The prevalence per 10,000 births was as follows: heterotaxia, 1.6; conotruncal defects, 11.6; atrioventricular septal defects, 5.6; anomalous pulmonary venous return, 1.1; left outflow obstructions, 8.7; right outflow obstructions, 5.6; septal defects, 65.5; isolated patent ductus arteriosus, 24.6; and other specified or unspecified CHD, 12.7. Excluding preterm patent ductus arteriosus, the CHD prevalence was 123.4 per 10,000; per year, the prevalence increased with 3.5% (95% CI 2.5-4.4) in 1994-2005 and declined with 9.8% (–16.7 to –2.4) from 2005 onwards. Severe CHD prevalence was 30.7 per 10,000; per-year increase was 2.3% (1.1-3.5) in 1994-2004, and per-year decrease was 3.4% (–6.6 to –0.0) in 2004-2009. Numbers included severe CHD in stillbirths and terminated pregnancies.

Conclusions The birth prevalence of CHD declined from around 2005. Specifically, the prevalence of severe CHD was reduced by 3.4% per year from 2004 through 2009. (Am Heart J 2014;168:956-64.)

Congenital heart defects (CHDs) are the most common birth defects, reported to affect 5 to 10 per 1,000 live births.[1-5] These cardiac anomalies vary from minor lesions without clinical significance to severe conditions requiring extensive health care and with impaired physical capacity and life expectancy. There is substantial variation in the reported CHD prevalence by year of birth and in different populations.[5] Reliable prevalence estimates are important tools in health care planning, as follow-up through adulthood is necessary for many children with CHD and repeated surgical procedures are often required. Because the etiology of CHD is largely unknown, time trends and changing prevalence in different populations might also give clues to differences in risk factors.[6]

During the 1980s and 1990s, the recorded birth prevalence of CHD increased substantially.[3,5,7] Improved diagnostic tools, such as high-quality ultrasound technology, may have led to increased detection of the mild anomalies in this time period. Although the septal defects accounted for the largest proportion of the overall increase in CHD prevalence,[3,7,8] several studies also reported an increased prevalence of severe heart defects.[3,4,6] However, recent studies have reported that the CHD trend is changing. In Quebec, Canada,[6] the prevalence of severe heart defects started to decrease from 1999. The authors suggested a preventive effect of mandatory folic acid fortification of cereal products introduced in Canada in 1998. In Atlanta, GA, however, where folic acid fortification of flour was introduced at the same time as in Canada, the CHD prevalence continued to increase until 2005.[7] Interestingly, in European countries where there has been no mandatory

From the [a]Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway, [b]Department of Heart Disease, Haukeland University Hospital, Bergen, Norway, [c]Department of Pediatrics, Haukeland University Hospital, Bergen, Norway, [d]Department of Medical Science, University of Bergen, Bergen, Norway, [e]Department of Pediatrics, Oslo University Hospital, Rikshospitalet, Oslo, Norway, [f]Division of epidemiology, Norwegian Institute of Public Health, Bergen, Norway, and [g]Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway.

The study was funded by Research Council Norway project number 190858/V50 to Dr Nina Øyen.

Submitted February 6, 2014; accepted July 16, 2014.

Reprint requests: Elisabeth Leirgul, MD, Department of Global Public Health and Primary Care, University of Bergen, PO box 7804, N-5020 Bergen, Norway.

E-mail: elisabeth.leirgul@uib.no

0002-8703

© 2014 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

http://dx.doi.org/10.1016/j.ahj.2014.07.030

food fortification with folic acid,[9] a study based on the European Surveillance of Congenital Anomalies (EUROCAT) registries 1990-2007[4] found a downward trend in heart defect birth prevalence from 2004 onwards. A shift from severe CHD in live births to severe CHD in terminated pregnancies because of increasing availability of prenatal diagnostics[4] could also have contributed to the drop in live birth prevalence of severe heart defects.

Norway's national health registers afford us the opportunity to conduct a nationwide study of prevalence and time trends of CHDs in live births, stillbirths, and terminated pregnancies.

Methods

Data sources

The Norwegian Population Register has since 1965 registered demographic data and vital status on all residents. The unique personal identification number enables linkage of data between national registries and other data sources.

The Medical Birth Registry of Norway (MBRN) was established in 1967 and has since then recorded information on all births (live births and stillbirths from 16th week of gestation, from 1999 also including terminated pregnancies, and from 2002 all births from 12th week of gestation),[10,11] including medical information of the mothers' health before and during pregnancy, the course of delivery, and the health of the newborn child. The MBRN contains information on CHDs diagnosed shortly after birth based on reports from maternity and pediatric wards.

The National Hospital's clinical database for children with heart disease contains information on all children with a heart condition who have been examined by a pediatric cardiologist or have received surgery or intervention at the National Hospital since 1992.[12] The National Hospital conducted about 80% of congenital heart surgeries in Norway before 2004 and virtually all thereafter.[13]

The multipurpose research project Cardiovascular Disease in Norway[14] established in collaboration between the University of Bergen and the Norwegian Knowledge Centre for the Health Services has retrieved information on cardiovascular diseases including CHDs from the electronic Patient Administrative System (PAS) of all somatic hospitals in Norway, 1994-2009.[15] Up to 20 discharge diagnoses for each hospital stay, as well as information about diagnostic and surgical procedures, are included in the database.

The Cause of Death Registry contains information about the underlying cause of death and up to 6 contributing causes of death, as recorded from the death certificate.

Study population

All births in Norway registered in The Medical Birth Registry from 1994 through 2009 were followed until 31 December 2009 for information on CHDs registered in the hospitals' PAS and until 31 December 2010 for the Medical Birth Registry, the National Hospital's clinical database for children with heart disease, and the Cause of Death Registry.

Case ascertainment and classification of CHDs

Information on individuals with CHD was ascertained from 4 data sources: (1) the National Hospital's clinical database for children with heart disease by van Mierop codes 100, 110, 120, 200, 210, 1002 to 7442[16,17] and *International Classification of Diseases 10th Revision* (ICD-10) codes Q20.0 to Q26.9, Q89.3; (2) the PAS by ICD-9 codes 745.0 to 747.4, 759.3 from 1994 through 1998, and ICD-10 codes Q20.0 to Q26.9, Q89.3 from 1999 onwards; (3) the Medical Birth Registry by ICD-8 codes 746.0 to 747.4, 759.0 from 1994 through 1997, and ICD-10 Q20.0 to Q26.9, Q89.3 from 1997 onwards; and (4) the Cause of Death Registry by ICD-9 745.0 to 747.4, 759.3 from 1994 through 1995, and ICD-10 Q20.0 to Q26.9, Q89.3 from 1996 onwards.

According to this procedure, we identified 16,365 children with codes for CHD. Among them, we excluded heart conditions likely to be physiologically normal, such as 1,727 cases with untreated isolated patent ductus arteriosus (PDA) without mention of the diagnosis after 6 weeks postnatal age; 1,061 untreated isolated atrial septal defects (ASDs) without recording of the diagnosis after 6 weeks (likely persistent foramen ovale); 151 isolated valvular pulmonary stenoses (vPS) without the diagnosis after 6 weeks (likely normal high flow in the pulmonary artery), and 344 cases with a combination of these conditions, leaving 13,082 children with CHD for phenotype classification.

Most children with CHD were registered in several data sources: 36.5% in 2 sources, 14.9% in 3 sources, and 1.2% in 4 sources. When assigning fetuses or children with CHD into cardiac phenotypes, diagnosis codes were first selected from the National Hospital's clinical database (48.0% of the children), then the first entry in the PAS (36.1%), with the priority for diagnoses from the university hospitals with facilities for pediatric cardiac surgery and invasive procedures, the Medical Birth Registry (15.2%), and the Cause of Death Registry (0.7%).

Children with CHD codes were classified into cardiac phenotypes as used by Botto et al,[2,18,19] Øyen et al,[2] and recently by Liu et al,[20] listing the diagnoses in order of priority as follows: *heterotaxia* with or without any other heart defect; *conotruncal defect* (d-transposition of the great arteries [TGA] or tetralogy of Fallot [ToF] with or without atrioventricular septal defect [AVSD], double outlet right ventricle [DORV], conoventricular ventricle septum defect [VSD], pulmonary atresia [PA], or valvular pulmonary atresia [vPA] with VSD [regarded as ToF anatomy], truncus arteriosus, interrupted aortic arch [IAA] type B or type C); *AVSD* with or without anomalous

pulmonary venous return (APVR); *APVR* including total or partial APVR; *left ventricle outflow tract obstruction (LVOTO)* (coarctation of aorta [CoA] or aortic valve stenosis [vAS] with or without VSD, hypoplastic left heart syndrome [HLHS]); *right ventricle outflow tract obstruction (RVOTO)* (hypoplastic right heart syndrome [HRHS], tricuspid atresia, Ebstein anomaly, PA or PvA with intact ventricular septum, and vPS with or without septal defects]; *septal defects* (VSD only, ASD only and recorded from postnatal age 6 weeks, VSD and ASD only); *other complex heart defect* (single ventricle, congenital corrected transposition of the great arteries); *PDA* at postnatal age >6 weeks or with surgical correction, in live births with gestational age ≥37 weeks, or with gestational age <37 weeks (preterm PDA); *other specified heart defect* (eg, isolated valvular malformations not classified as LVOTO or RVOTO and venous malformations); and *unspecified heart defect*. To the last category, we added 89 children who had been registered with diagnosis codes for lethal conditions and alive at age 2 years, but without registered heart surgery or invasive procedure, assuming the heart defect was misclassified.

Severe CHD was defined as heterotaxia, conotruncal defect, AVSD, APVR, LVOTO, RVOTO (except valvular pulmonary stenosis), or other complex heart defect. In a subanalysis, VSDs were divided into those corrected with a percutaneous or surgical procedure and those without such procedures.

Information on chromosome aberrations was retrieved using ICD codes (eighth revision 759.3-759.5, ninth revision 758.0-759.9, 10th revision D82.1, Q90.0-Q99.9) and van Mierop codes (8000-8004, 8009-8025, 8072) in all data sources. In addition, we searched for notes indicating chromosomal disorders in text entry fields in the National Hospital's clinical database for children with heart disease, and Down syndrome registered in the Medical Birth Registry of Norway. The CHD children with extracardiac malformations were identified by ICD codes (eighth revision 740.0-745.9, 748.0-756.9, 759.8-759.9, ninth revision 740.0-744.9, 748.0-756.9, 759.0-759.9, 10th revision Q00.0-Q18.9, Q30.0-Q89.9) and van Mierop codes (8041-8053, 8066, 8074-8076, 8079, 8099), in addition to selected congenital malformations registered in MBRN. The categories chromosomal aberrations and extracardiac malformations were not mutually exclusive.

The age at diagnosis was set to 0 day if the cardiac defect was reported from the maternity ward, otherwise, the age at the first hospitalization reporting CHD, or the age at a cardiac procedure.

Statistical analysis

Birth prevalence was reported as number of persons affected with CHD per 10,000 births (live births, stillbirths, and terminated pregnancies) for the entire period 1994-2009. Then, the prevalences of severe defects combined, VSD, ASD, and PDA were calculated by year of birth. Next, we modeled annual CHD prevalence with The National Cancer Institute's Joinpoint Regression Program version 4.0.4[7,21] to estimate annual percent change (APC) with 95% CIs using the best fitting model. And finally, the yearly prevalence of severe defects was stratified on type of birth, that is, live birth (singleton or multiple birth) with isolated severe heart defect, live birth with severe heart defect and extracardiac defect, stillbirth with severe heart defect, and terminated pregnancy with diagnosed severe heart defect. The median age at diagnosis was calculated for the period 1994-2009 for severe CHD, ASD, and VSD. Cumulative percentile curves for diagnosis were calculated at age 1, 7, 30, 180, 365, and 730 days for severe CHD, and VSD, for the period 2004-2008. The data linkage and all calculations were performed with SAS (version 9.3; SAS Institute Inc, Cary, NC).

Results

Among 954,413 births registered in the Medical Birth Registry 1994-2009, we identified 13,081 individuals with one or more CHDs; the overall birth prevalence was 137.1 per 10,000 (Table I). After excluding isolated PDA in preterm births (gestational age <37 weeks), the birth prevalence of CHD was 123.4 per 10,000. Congenital heart defects were more frequent in girls, with birth prevalence of CHD 136.2 per 10,000 (excluding preterm PDA 123.3 per 10,000), compared to 131.1 per 10,000 (excluding preterm PDA 116.6 per 10,000) in boys ($P < .05$). Severe CHD was found in 2,930 births, with an overall prevalence of 30.7 per 10,000 births and 28.3 per 10,000 live births.

The most common cardiac defects were isolated septal defects; the birth prevalence was 65.5 per 10,000 (Table I). Around three-quarters of these were VSDs, with 48.4 per 10,000 births. The prevalence of conotruncal defects was 11.6 per 10,000; the most frequent was TGA, 3.5, and ToF, 2.7 per 10,000. Left ventricular outlet tract obstructions accounted for 8.7 per 10,000, including HLHS, 2.6, vAS, 3.0, CoA, 3.0, and IAA type A, 0.1 per 10,000, and right ventricular outlet tract obstructions 5.6 per 10,000, among these HRHS, 0.9, Ebstein anomaly, 0.7, and vPS, 3.6 per 10,000. The prevalence of AVSD was 5.6, of APVR 1.1, and of heterotaxia 1.6 per 10,000 births. Other complex heart defects were found in 0.2 per 10,000. Isolated PDA had a prevalence of 24.6 per 10,000. In term births, prevalence of isolated PDA was 10.9 per 10,000. Other specified CHD was found in 6.6, and unspecified CHD in 6.1 per 10,000 births.

The live birth prevalence of CHD was 133.2 per 10,000 and excluding preterm PDA 119.4 per 10,000, whereas the prevalence of CHD among stillbirths or pregnancies terminated for medical reasons was more than 3 times as high, with a prevalence of 478.1 per 10,000 (Table I). Specific cardiac phenotypes, such as heterotaxia,

Table I. Prevalence of CHDs in Norway, 1994-2009

Heart defect phenotype	All births n = 954413		Live births n = 943871		Stillbirths/terminated pregnancies n = 10542	
	n	Prevalence*	n	Prevalence†	n	Prevalence‡
Any CHD	13081	137.1	12577	133.2	504	478.1
CHD excl. preterm PDA	11776	123.4	11272	119.4	504	478.1
Heterotaxia	149	1.6	133	1.4	16	15.2
Conotruncal defect	1110	11.6	1040	11.0	70	66.4
TGA	331	3.5	308	3.3	23	21.8
ToF	258	2.7	243	2.6	15	14.2
DORV	79	0.8	66	0.7	13	12.3
Other conotruncal§	442	4.6	423	4.5	19	18.0
AVSD	530	5.6	492	5.2	38	36.0
APVR	107	1.1	107	1.1	0	0.0
LVOTO	830	8.7	722	7.6	108	102.4
HLHS	244	2.6	154	1.6	90	85.4
CoA	288	3.0	273	2.9	15	14.2
Valv. aortic stenosis	290	3.0	287	3.0	3	2.8
IAA A	8	0.1	8	0.1	0	0.0
RVOTO	532	5.6	506	5.4	26	24.7
HRHS	82	0.9	66	0.7	16	15.2
Ebstein	64	0.7	57	0.6	7	6.6
vPS	348	3.6	347	3.7	1	0.9
Other RVOTO‖	38	0.4	36	0.4	2	1.9
Septal defect, isolated	6248	65.5	6113	64.8	135	128.1
ASD	1350	14.1	1342	14.2	8	7.6
VSD	4620	48.4	4506	47.7	114	108.1
VSD + ASD	189	2.0	179	1.9	10	9.5
Unsp. septal defect	89	0.9	86	0.9	3	2.8
Other complex CHD	20	0.2	20	0.2	0	0.0
Isolated PDA¶	2345	24.6	2345	24.8	0	0.0
At term gestation	1040	10.9	1040	11.0	0	0.0
Preterm gestation	1305	13.7	1305	13.8	0	0.0
Other specified CHD	632	6.6	597	6.3	35	33.2
Unspecified CHD	578	6.1	502	5.3	76	72.1
Associations:						
Conotruncal + AVSD	16	0.2	15	0.2	1	0.9
Septal + LVOTO	79	0.8	78	0.8	1	0.9
Septal + RVOTO	99	1.0	99	1.0	0	0.0

* Prevalence per 10,000 births (live births, stillbirths, terminated pregnancies) registered in the Medical Birth Registry.
† Prevalence per 10,000 live births.
‡ Prevalence per 10,000 stillbirths and terminated pregnancies.
§ Truncus arteriosus, conotruncal VSD, aortopulmonary window, IAA type B or C.
‖ Valvular pulmonary atresia, arterial pulmonary atresia.
¶ PDA recorded after 6 weeks of age or surgically treated.

conotruncal defects, AVSD, and HLHS, showed very high prevalence in stillbirths/terminated pregnancies.

In Table II, the distribution of cardiac phenotypes was shown for multiple birth pregnancies, in children with a chromosomal aberration, and in children with extracardiac birth defects. Among all births with CHD, 8.6% was part of a multiple-birth pregnancy, 10.6% had been diagnosed with a chromosomal aberration, and 21.0% had extracardiac defects. For example, chromosomal aberrations were frequent with AVSD, and extracardiac defects with heterotaxia and conotruncal defects.

In Figure 1, the prevalences of severe CHD, ASD, VSD, PDA, and the remaining defects (vPS, other specified CHD, and unspecified CHD) were presented by year of birth. From 1994 until the beginning of the 2000s, all defects increased in prevalence. Ventricular septal defects had the most marked increase, with roughly doubled prevalence from 1994 to 2007. The prevalence of repaired VSD was stable with a mean prevalence of 5.5 per 10,000 throughout the entire study period 1994-2009 (not shown in figure). Median age at diagnosis for ASD was 323 days; whereas for VSD, PDA, and severe CHD, it was 0 days, that is, diagnosed before leaving the maternity ward. The VSD diagnosis was registered before age 30 days in 92% of the cases, before 6 months in 97%, and within the first year of life in 98% of the VSDs. For severe defects, the

Table II. Multiple birth and extracardiac defects in children with CHD, Norway 1994-2009*

Heart defect phenotype	All births	Multiple birth		Chromosomal aberrations		Extracardiac malformations	
	n	n	(%)	n	(%)	n	(%)
Any CHD	13081	1123	(8.6)	1389	(10.6)	2746	(21.0)
CHD excl. preterm PDA	11776	688	(5.8)	1365	(11.6)	2487	(21.1)
Heterotaxia	149	7	(4.7)	17	(11.4)	135	(90.6)
Conotruncal defect	1110	45	(4.1)	172	(15.5)	349	(31.4)
TGA	331	4	(1.2)	21	(6.3)	66	(19.9)
ToF	258	15	(5.8)	50	(19.4)	89	(34.5)
DORV	79	2	(2.5)	12	(15.2)	38	(48.1)
Other conotruncal†	442	24	(5.4)	89	(20.1)	156	(35.3)
AVSD	530	23	(4.3)	299	(56.4)	134	(25.3)
APVR	107	4	(3.7)	7	(6.5)	33	(30.8)
LVOTO	830	50	(6.0)	89	(10.7)	176	(21.2)
HLHS	244	9	(3.7)	18	(7.4)	47	(19.3)
CoA	288	25	(8.7)	42	(14.6)	70	(24.3)
Aortic stenosis	290	16	(5.5)	28	(9.7)	58	(20.0)
IAA A	8	0	(0.0)	1	(12.5)	1	(12.5)
RVOTO	532	31	(5.8)	35	(6.6)	125	(23.5)
HRHS	82	4	(4.9)	6	(7.3)	26	(31.7)
Ebstein	64	0	(0.0)	3	(4.7)	9	(14.1)
vPS	348	25	(7.2)	24	(6.9)	82	(23.6)
Other RVOTO‡	38	2	(5.3)	2	(5.3)	8	(21.1)
Septal defect, isolated	6248	390	(6.2)	582	(9.3)	1048	(16.8)
ASD	1350	110	(8.1)	183	(13.6)	326	(24.1)
VSD	4620	264	(5.7)	317	(6.9)	641	(13.9)
VSD + ASD	189	10	(5.3)	77	(40.7)	75	(39.7)
Unsp. septal defect	89	6	(6.7)	5	(5.6)	6	(6.7)
Other complex CHD	20	0	(0.0)	1	(5.0)	6	(30.0)
Isolated PDA§	2345	473	(20.2)	81	(3.5)	471	(20.1)
At term gestation	1040	38	(3.7)	57	(5.5)	212	(20.4)
Preterm gestation	1305	435	(33.3)	24	(1.8)	259	(19.8)
Other specified CHD	632	65	(10.3)	37	(5.9)	128	(20.3)
Unspecified CHD	578	35	(6.1)	69	(11.9)	141	(24.4)
Associations:							
Conotruncal + AVSD	16	0	(0.0)	6	(37.5)	6	(37.5)
Septal + LVOTO	79	8	(10.1)	18	(22.8)	25	(31.6)
Septal + RVOTO	99	5	(5.1)	7	(7.1)	29	(29.3)

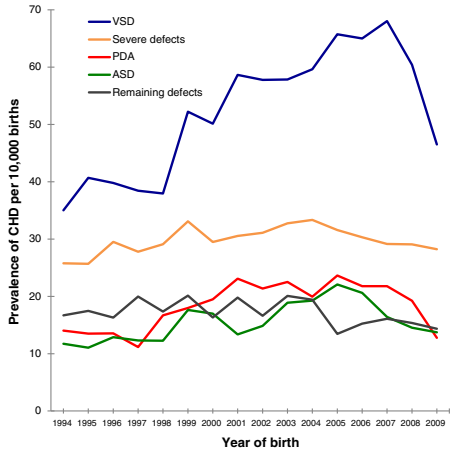
* All live births, stillbirths, and terminated pregnancies registered in the Medical Birth Registry.
† Truncus arteriosus, conotruncal VSD, aortopulmonary window, IAA type B or C.
‡ Valvular pulmonary atresia, arterial pulmonary atresia.
§ PDA recorded after 6 weeks of age or surgically treated.

corresponding cumulative percentages for ages at diagnosis were 85%, 94%, and 95%, respectively.

In Figure 2, we plotted the prevalence of any type CHD (excluding preterm PDA), severe CHD, VSD, and ASD for all births, including stillbirths and terminated pregnancies. Using the Joinpoint Regression Program, the best fit for time trend change was identified. Among all births, the annual increase in total CHD prevalence was 3.5% (95% CI 2.5-4.4) until 2006; thereafter, the prevalence decreased with 9.8% per year (−16.7 to −2.4). Severe CHD prevalence showed an annual increase of 2.3% (1.1-3.5) until 2004 and an annual decrease of 3.4% (−6.6 to 0.0) thereafter. The prevalence of ASD increased with 5.7% (3.2-8.3) per year until 2005 and then decreased with 12.0% (−21.3 to −1.9) per year. Ventricular septal defect prevalence increased with 5.4% (4.0-6.8) per year until 2007 and then showed a marked but nonsignificant annual decrease of 18.6% (−36.7 to 4.6) the last 2 years of follow-up. Among live births only (not shown in the figure), the APC for any type CHD was 3.5% (2.4-4.7) until 2005 and −7.5% (−12.2 to −2.4) thereafter; and for severe CHD, the APC was 1.8% (0.2-3.4) until 2003 and −4.6% (−7.3 to −1.8) thereafter. The time trend curve for severe CHD in live births only did not differ much from the severe CHD time trend in all births.

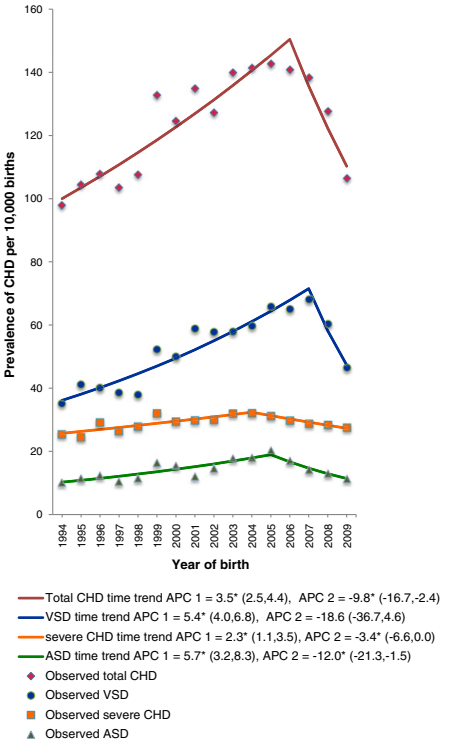
In Figure 3, the annual birth prevalence of severe CHD in Figure 2 was classified into combinations of birth type, plurality (singletons, multiples), and the presence of extracardiac birth defects and/or chromosomal aberrations. The live birth prevalence of singletons with severe

Figure 1



Birth prevalence of CHD per 10,000 live births, stillbirths, and terminated pregnancies by year of birth for isolated VSD, severe defects (heterotaxia, conotruncal defect, AVSD, APVR, LVOTO, RVOTO except valvular pulmonary stenosis, CoA, other complex heart defect), PDA, isolated ASD, and all other remaining defects in 954,413 births, Norway, 1994-2009.

Figure 2



Birth prevalence of CHD per 10,000 live births, stillbirths, and terminated pregnancies by year of birth for all CHD (excluding isolated preterm PDA), VSD, ASD, and severe heart defects (heterotaxia, conotruncal defect, AVSD, APVR, LVOTO, RVOTO except valvular pulmonary stenosis, CoA, other complex heart defect) in 954,413 births, Norway, 1994-2009. Observed and estimated prevalence, and APC with 95% CI, using Joinpoint Regression Program.[21] *$P < .05$.

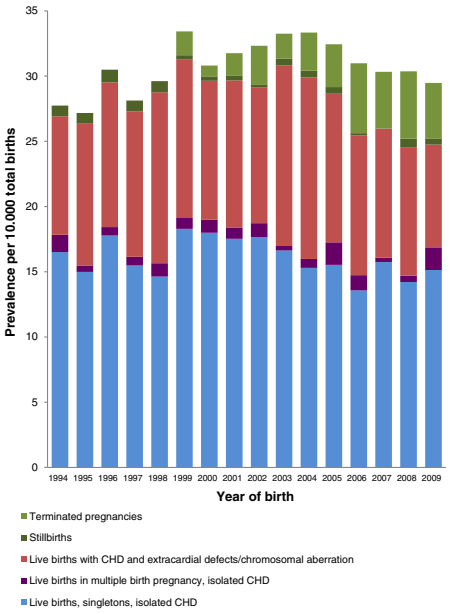
CHD but without extracardiac defects or chromosomal disorders was around 15 per 10,000 births, with a decreasing trend from around 1999 (blue color). Next, the live birth prevalence of twins or triplets with isolated CHD constituted a very small fraction of the total severe CHD prevalence (purple color). The prevalence of live births with severe CHD and extracardiac defects/chromosomal disorders ranged 10 to 14 per 10,000 (red color). Finally, the prevalences for severe CHD in stillbirths and terminated pregnancies increased from 0.9 per 10,000 total births in 1994-1997 to 1.6 in 1998-2001, 3.2 in 2002-2005, and 5.1 in 2006-2009; the overall severe CHD prevalence was 2.7 per 10,000 births (green color). The proportion of terminated pregnancies and stillbirths in births with severe CHD increased from 1994 through 2009; among the total numbers of severe CHD, affected stillbirths and terminated pregnancies combined (n = 257) constituted 8.8%: 3.0% in 1994-1997, 5.0% in 1998-2001, 9.8% in 2002-2005, and 16.9% in 2006-2009 ($P < .001$).

Discussion

In this nationwide study of CHDs, we identified >13,000 individuals with a CHD among 954,500 live births, stillbirths, and terminated pregnancies registered in the Medical Birth Registry of Norway from 1994 to 2009, yielding a national CHD prevalence of 137.1 per 10,000. There was a significant increase in the prevalence of CHD until 2005, followed by a significant decrease in prevalence, both for total CHD and for severe CHD.

The overall prevalence of CHD in the present study was higher than that reported in similar studies from Denmark, other European countries, and Atlanta, GA.[3,4,7] In the Danish study,[3] the overall prevalence excluding isolated PDA in preterm infants was 103.2 per 10,000 live births versus 123.4 in our study. It is primarily the prevalence of septal defects, particularly VSD, that was higher in the present study, whereas the overall prevalence of severe defects was similar to our findings. The Danish study also reported an increasing prevalence

Figure 3



Birth prevalence of severe CHD by year of birth, stratified on type of birth (live birth, stillbirth/terminated pregnancies, singleton/multiple birth), and type of cardiac defects (isolated or accompanied with extracardiac birth defects and/or chromosomal aberration) in 954,413 births, Norway, 1994-2009. Severe heart defects were heterotaxia, conotruncal defect, AVSD, APVR, LVOTO, RVOTO (except valvular pulmonary stenosis), and other complex heart defects.

of severe heart defects from 1977 to 2005, like the present study. A Canadian study,[6] which included children born in Quebec with selected severe CHDs (ToF, endocardial cushion defects, univentricular hearts, truncus arteriosus, transposition complexes) in the period 1990-2005, reported a prevalence of 15.7 per 10,000 births, which is similar to the prevalence of the corresponding defects in our study and in Denmark. However, the study from EUROCAT, covering 29% of the European birth population, reported considerably lower prevalence of the severe CHD (single ventricle, HLHS, HRHS, Ebstein anomaly, tricuspid atresia, vPA, truncus arteriosus, AVSD, ToF, vAS, TGA, TAPVR, CoA), 17.7 per 10,000,[4] as compared to the present study, 30.7 per 10,000. Although the severe CHD prevalence was lower in the EUROCAT study, the pattern of time trends was similar to the present study with an increase from 1990 to 2004 and a decrease thereafter. The study from Atlanta[7] also reported lower birth prevalence of total CHD and severe CHD, 67.7 and 24.9 per 10,000, respectively, in the period 1978-2005.

As suggested in the previous studies,[3,7,8] the increasing prevalence of minor CHD may be partly explained by improved diagnostic equipment. This applies primarily to the period before year 2000. The continued increase of both minor and severe CHD until 2006, however, has most likely other explanations. We cannot rule out that the CHD prevalence increase could partially be explained by improved reporting of birth defects. In 1999, the Medical Birth Registry implemented a revised notification form, which may have led to higher quality of birth defect reporting, including both mild and severe CHD. Mandatory reporting to MBRN of terminated pregnancies was implemented: from 1999 to 2001 for pregnancies after 16th gestational week and from 2002 after 12th gestational week.[11] This can explain some of the increase in severe CHD from 1994 to 2004.

After the mid-2000s, the prevalence of CHD declined, most markedly for VSD; but also severe defect prevalence declined. The prevalence of CHD could have been underestimated in the last period because of shorter follow-up time. However, VSD demonstrates a distinct murmur usually detected at the maternity ward or the child health clinic, also reflected in our study; 94% of the VSDs had been diagnosed before age 6 months. The large VSD decline the last 2 years of the study period likely represented a true decrease. Most severe defects are symptomatic in early life; in the present study, 94% were diagnosed before 6 months of age. Therefore, the declining prevalence of severe CHD from 2005 through 2009 cannot be fully explained by incomplete case ascertainment due to a shorter follow-up of births in the late period. We acknowledge that ASD, with a median age at diagnosis of 323 days, could have been underestimated in the last period.[3]

At present, the reasons for the decline of CHD prevalence in Norway from the mid-2000-ies are unknown. One explanation for the recent decrease in CHD prevalence in Norway, as in other European countries and Canada, could be an increased intake of folic acid in fertile women. In 1998, food fortification of folic acid was introduced in both Canada and Atlanta, GA, whereas in the European countries, including Norway, official authorities only recommended intake of folic acid supplements for women planning a pregnancy and early in pregnancy.[22] Prenatal vitamin supplementation policy in our study population has been unchanged since 1999. However, the uptake of the recommendations, reflected by an increasing use of preconception folic acid supplementation in Norway, from 5% to 26% in the period 1999-2007,[23] could explain the temporal decrease in CHD prevalence reported in our study, assuming a causal association between folic acid intake and CHD.[24] In Canada, the decreasing prevalence of severe CHD from 1999 coincided with the implementation of food fortification of folic acid. Interestingly, the severe CHD reduction was delayed in Europe starting from the mid-

2000s, which could be explained by a gradual increase of intake of supplements containing folic acid among women in Europe from the end of the 1990s.[4,22] However, in Atlanta, GA, the severe defect prevalence continued to increase until 2005[7] despite the mandatory folate fortification of staple food beginning in 1998.

Alternative explanations for the recent decreasing prevalence of severe CHD in Canada and Europe could be other preventive factors, for example, cessation of maternal smoking,[25] or better monitoring of women with diabetes[26] or other chronic diseases, or maybe a reduction in consanguineous marriages,[27] which are known to be associated with increased birth defect risk. However, in the present study, the births among first-generation immigrants with a high proportion of first-cousin marriage[28] amounted only to 1.1% of all births, with an overall CHD prevalence of 165.8 per 10,000 births. Therefore, a change in consanguineous marriages cannot explain the declining prevalence of CHD in Norway. Finally, we cannot rule out the possibility of random fluctuation; surveillance of CHD remains important in the future.

A limitation of the present study was a possible misclassification of diagnosis codes for CHD because reviewing the medical records for 13,000 patients was not feasible. Besides, we were not allowed by the Ethics Committee to investigate individual medical records. However, almost half of the cases and nearly all the severe defect cases were retrieved from the National Hospital's clinical database for children with heart disease (NHCD). Senior pediatric cardiologists have regularly entered and updated codes for heart defect diagnoses and procedures into this clinical database, which has minimized the possibility of misclassification of severe defects. Furthermore, in the PAS, we selected diagnosis codes from the university hospitals with facilities for pediatric cardiology surgery and invasive procedures. Finally, we cross-checked diagnosis codes in all 2,999 individuals notified with lethal conditions against their survival status at age 2 years and surgery or procedure codes, and identified only 89 (2.8%) misclassified severe defect cases, which we placed in the unspecified CHD category. Congenital heart defects with chromosomal aberrations and/or extra-cardiac defects could have been missed if the cardiac defect was not coded, for example, in stillbirths or terminated pregnancies. However, the proportions of chromosomal aberrations (10.6%) and extracardiac defects (21.0%) were comparable to findings from Denmark (7.0% and 22.3%).[3] There was a risk of including physiological heart conditions notified shortly after birth. To avoid this, untreated ASD, PDA, and vPS only recorded the first 6 postnatal weeks were excluded. Some minor CHDs could have been missed because outpatient clinics' data were only included from the National Hospital's clinical database for children with heart disease. Considering the high prevalence of minor heart defects in the present study, as compared with previous studies, we consider the possible missing outpatient minor defects of little importance.

The strength of the present study was the virtually complete registration of both severe and minor CHD, including cardiac defects in terminated pregnancies, ascertained through 4 national health and administrative registers. This is possible due to the unique personal identification number given shortly after birth, allowing follow-up through life. Because medical care for children is free in Norway and health care is highly developed, nearly all children with CHD are diagnosed at an early age. Finally, the hierarchical structure of our classification system assigning individuals with multiple heart defects to only one cardiac phenotype precluded counting these individuals several times, thus avoiding an overestimation of the total prevalence.

In conclusion, in the present population-based study, we found increasing prevalence of severe CHD from 1994 until 2005 and decreasing prevalence thereafter, corresponding to findings in European and Canadian studies. Although there was an increasing practice of pregnancy termination of fetuses with severe CHD, this contributed little to the time trends in CHD prevalence in Norway. The reasons for the downward change in time trends of CHD and severe CHD from the mid-2000s are unknown but seem related to factors not only changing in Norway. Suggested changes in maternal risk factors, such as an increasing use of folic acid supplementation, or better follow-up of pregnant women with chronic diseases like diabetes has been proposed. Further investigation is required to determine the effect of possible risk factors, as this can provide a basis for treatment, lifestyle advice, or public health interventions.

Acknowledgements

The authors thank Tomislav Dimoski, the Norwegian Knowledge Centre for Health Services, Oslo, Norway, for his contribution by developing the software necessary for obtaining data from Norwegian hospitals, conducting the data collection, and quality assurance of data in this project, and Dr Petter Hagemo for construction and maintenance of the clinical database for CHDs at the Oslo University Hospital, Norway.

Disclosures

The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the manuscript, and its final contents.

References

1. Hoffman JI, Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol 2002;39(12):1890-900.

2. Botto LD, Lin AE, Riehle-Colarusso T, et al. Seeking causes: classifying and evaluating congenital heart defects in etiologic studies. Birth Defects Res A Clin Mol Teratol 2007;79(10):714-27.
3. Øyen N, Poulsen G, Boyd HA, et al. National time trends in congenital heart defects, Denmark, 1977-2005. Am Heart J 2009;157(3): 467-473.e1.
4. Khoshnood B, Loane M, Garne E, et al. Recent decrease in the prevalence of congenital heart defects in Europe. J Pediatr 2013;162 (1):108-113.e2.
5. van der Linde D, Konings EE, Slager MA, et al. Birth prevalence of congenital heart disease worldwide: a systematic review and meta-analysis. J Am Coll Cardiol 2011;58(21):2241-7.
6. Ionescu-Ittu R, Marelli AJ, Mackie AS, Pilote L. Prevalence of severe congenital heart disease after folic acid fortification of grain products: time trend analysis in Quebec, Canada. BMJ 2009;338:b1673.
7. Bjornard K, Riehle-Colarusso T, Gilboa SM, Correa A. Patterns in the prevalence of congenital heart defects, metropolitan Atlanta, 1978 to 2005. Birth Defects Res A Clin Mol Teratol 2013;97(2):87-94.
8. Wren C, Richmond S, Donaldson L. Temporal variability in birth prevalence of cardiovascular malformations. Heart 2000;83(4):414-9.
9. Bitzer J, von Stenglin A, Bannemerschult R. Women's awareness and periconceptional use of folic acid: data from a large European survey. Int J Womens Health 2013;5:201-13.
10. Irgens LM. The Medical Birth Registry of Norway; a source for epidemiological and clinical research. Scand J Rheumatol Suppl 1998;107:105-8.
11. Folkehelseinstituttet. Annual report 2001-2002, Medical Birth Registry of Norway [cited 2004]. Available from, http://www.fhi.no/dav/f1065301e2.pdf.
12. Hagemo PS. BERTE—a database for children with congenital heart defects. Stud Health Technol Inform 1994;14:98-101.
13. Annual Report 2001-2002. Medical Birth Registry of Norway. 2004.
14. CVDNOR cardiovascular disease in Norway., https://cvdnor.b.uib.no.
15. Sulo GIJ, Vollset SE, Nygård O, et al. Cardiovascular disease and diabetes mellitus in Norway during 1994-2009 CVDNOR—a nationwide research project. Nor J Epidemiol 2013;23(1):101-7.
16. van Mierop LH. Diagnostic code for congenital heart disease. Pediatr Cardiol 1984;5(4):331-62.
17. Van Mierop LH. Diagnostic code for congenital heart disease, supplement. Pediatr Cardiol 1986;7(1):31-4.
18. Strickland MJ, Riehle-Colarusso TJ, Jacobs JP, et al. The importance of nomenclature for congenital cardiac disease: implications for research and evaluation. Cardiol Young 2008;18 (Suppl 2):92-100.
19. Riehle-Colarusso T, Strickland MJ, Reller MD, et al. Improving the quality of surveillance data on congenital heart defects in the metropolitan Atlanta congenital defects program. Birth Defects Res A Clin Mol Teratol 2007;79(11):743-53.
20. Liu S, Joseph KS, Lisonkova S, et al. Association between maternal chronic conditions and congenital heart defects: a population-based cohort study. Circulation 2013;128(6):583-9.
21. Kim HJ, Fay MP, Feuer EJ, Midthune DN. Permutation tests for joinpoint regression with applications to cancer rates. Stat Med 2000;19(3):335-51.
22. Daltveit AK, Vollset SE, Lande B, Oien H. Changes in knowledge and attitudes of folate, and use of dietary supplements among women of reproductive age in Norway 1998-2000. Scand J Public Health 2004;32(4):264-71.
23. Folkehelseinstituttet. Facts on folic acid supplementation and pregnancy. Available from, http://www.fhi.no/artikler/?id=513982013.
24. Czeizel AE, Dudas I, Vereczkey A, Banhidy F. Folate deficiency and folic acid supplementation: the prevention of neural-tube defects and congenital heart defects. Nutrients 2013;5(11):4760-75.
25. Alverson CJ, Strickland MJ, Gilboa SM, Correa A. Maternal smoking and congenital heart defects in the Baltimore-Washington Infant Study. Pediatrics 2011;127(3):e647-53.
26. Lisowski LA, Verheijen PM, Copel JA, et al. Congenital heart disease in pregnancies complicated by maternal diabetes mellitus. An international clinical collaboration, literature review, and meta-analysis. Herz 2010;35(1):19-26.
27. Grjibovski AM, Magnus P, Stoltenberg C. Decrease in consanguinity among parents of children born in Norway to women of Pakistani origin: a registry-based study. Scand J Public Health 2009;37(3): 232-8.
28. Surén P, Grjibovski AM, Stoltenberg C. Consanguinity in Norway. Extent and medical consequences. Folkehelseinstituttet. 20072.

III


Paediatric and Perinatal Epidemiology

doi: 10.1111/ppe.12212

Periconceptional Folic Acid Supplementation and Infant Risk of Congenital Heart Defects in Norway 1999–2009

Elisabeth Leirgul,[a,b] Trude Gildestad,[a] Roy Miodini Nilsen,[a,c] Tatiana Fomina,[a] Kristoffer Brodwall,[a] Gottfried Greve,[b,d] Stein Emil Vollset,[a,e] Henrik Holmstrøm,[f] Grethe S. Tell,[a,e] Nina Øyen[a,g]

[a]*Department of Global Public Health and Primary Care*

[d]*Department of Clinical Science, University of Bergen*

[b]*Department of Heart Disease, Haukeland University Hospital*

[c]*Department of Clinical Research, Haukeland University Hospital*

[e]*Norwegian Institute of Public Health*

[g]*Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen*

[f]*Department of Pediatrics, Oslo University Hospital, Oslo, Norway*

Abstract

Background: The birth prevalence of congenital heart defects (CHDs) has decreased in Canada and Europe. Recommended intake of folic acid in pregnancy is a suggestive risk-reducing factor for CHDs. We investigated the association between periconceptional intake of folic acid supplements and infant risk of CHDs.

Methods: Information on maternal intake of folic acid supplements before and during pregnancy in the Medical Birth Registry of Norway 1999–2009 was updated with information on CHD diagnoses from national health registers and the Cardiovascular Diseases in Norway Project. The association between folic acid intake and infant risk of CHD was estimated as relative risk (RR) with binomial log linear regression.

Results: Among 517 784 non-chromosomal singleton births, 6200 children were identified with CHD and 1153 with severe CHD. For all births, 18.4% of the mothers initiated folic acid supplements before pregnancy and 31.6% during pregnancy. The adjusted RR for severe CHD was 0.99 [95% confidence interval [CI] 0.86, 1.13] comparing periconceptional intake of folic acid with no intake. Specifically, RR for conotruncal defects was 0.99 [95% CI 0.80, 1.22], atrioventricular septal defects 1.19 [95% CI 0.78, 1.81], left ventricular outflow tract obstructions 1.02 [95% CI 0.78, 1.32], and right ventricular outflow tract obstructions 0.97 [95% CI 0.72, 1.29]. Birth prevalence of septal defects was higher in the group exposed to folic acid supplements with RR 1.19 [95% CI 1.10, 1.30].

Conclusions: Periconceptional folic acid supplement use showed no association with severe CHDs in the newborn. An unexpected association with an increased risk of septal defects warrants further investigation.

Keywords: *Norway, cohort study, folic acid supplementation, congenital heart defects, epidemiology.*

Introduction

Congenital heart defects (CHDs) are among the most common birth defects and represent an important cause of infant morbidity and mortality, affecting 5–13 per 1000 births worldwide.[1–3] After decades of increasing prevalence of CHDs believed to be explained by improved diagnostics and reporting,[4,5] several studies have shown a changing time trend with decreasing prevalence in Canada and Europe after 1999,[6,7] and also in Norway from 2005.[3] This decline in CHDs has been partly explained by the concurrent introduction of folic acid food fortification or the increasing use of periconceptional folic acid supplements. Folic acid is the synthetic form of folate, which is necessary in DNA, RNA, and protein synthesis, and therefore important during fetal development. Periconceptional intake of folic acid has been shown to reduce the risk for neural tube defects,[8,9] and women worldwide have been recommended to take folic acid supplements before conception and in the beginning of pregnancy. More than 70 countries have also implemented folic acid fortification of grain products,[5,6] whereas health authorities in Norway have refrained from such fortification and recommended periconceptional use of

Correspondence:
Elisabeth Leirgul, Department of Global Public Health and Primary Care, University of Bergen, PO box 7804, NO-5020 Bergen, Norway.
E-mail: elisabeth.leirgul@uib.no

© 2015 The Authors. Paediatric and Perinatal Epidemiology Published by John Wiley & Sons Ltd.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

folic acid supplements.[10] A possible protective effect of folic acid supplements on CHDs is, however, controversial. While some studies have reported reduced risk of CHDs in children whose mothers have taken multivitamins with folic acid[11,12] or pure folic acid supplements,[13,14] or after folic acid food fortification,[6] other studies have reported no effect on CHDs of folic acid food fortification.[15,16]

In the Medical Birth Registry of Norway, information on the use of folic acid and multivitamin supplements has been recorded for all women giving birth since 1999.[17] We have taken advantage of Norway's national health registries and the Cardiovascular Disease in Norway project (CVDNOR) to investigate infant risk of specific types of CHDs among mothers using folic acid supplements in the periconceptional period.

Methods

Data sources

The Norwegian National Registry contains demographic data and vital status on all residents since 1965. Every resident's unique personal identification number enables linkage of data between national registries and other data sources. The Medical Birth Registry of Norway has since 1967 recorded information on all births (livebirths and stillbirths, since 1999 terminated pregnancies for fetal reasons, and since 2002 all births from 12th week gestation) on the birth notification form, including information on the mother's health, the course of the pregnancy and delivery, and the health of the newborn.[17] Oslo University Hospital's clinical registry for children with heart disease has registered all children with a heart defect admitted to Oslo University Hospital since 1992.[18] The multipurpose research project CVDNOR[19] includes information on discharge diagnoses retrieved from the electronic Patient Administrative Systems of all somatic hospitals in Norway 1994–2009.[19] The Cause of Death Registry records contributing causes of death from death certificates. Statistics Norway provides demographic data for all residents, including educational level, occupation, income, and marital status.[20]

Case ascertainment and classification of congenital heart defects

Information on CHD diagnoses was retrieved from the four data sources mentioned above, as published previously,[3] where each child was assigned a cardiac phenotype with priority corresponding to the timing of presumed errors in fetal heart development[2,3]: *heterotaxia*; *conotruncal defects* [d-transposition of the great arteries (TGAs), tetralogy of Fallot (ToF), double outlet right ventricle, conoventricular ventricle septal defect (VSD), common arterial trunk, interrupted aortic arch (IAA) type B or C]; *atrioventricular septal defect* (AVSD); *anomalous pulmonary venous return* (APVR); *left ventricle outflow tract obstruction* (LVOTO) [coarctation of the aorta (CoA), aortic valve stenosis (vAS), hypoplastic left heart syndrome (HLHS)]; *right ventricle outflow tract obstruction* (RVOTO) [hypoplastic right heart syndrome (HRHS), tricuspid atresia, Ebstein anomaly, pulmonary atresia (PA) or atresia of the pulmonary valve with intact ventricular septum, valvular pulmonary stenosis (vPS)]; *septal defect* [VSD only, atrial septal defect (ASD) only and recorded from postnatal age 6 weeks or with surgical or percutaneous correction, VSD and ASD only]; *other complex heart defect* [single ventricle, congenital corrected transposition of the great arteries (ccTGA)]; *patent ductus arteriosus* (PDA) at postnatal age > 6 weeks or with surgical correction, in livebirths with gestational age 37 weeks or more (term PDA), or with gestational age < 37 weeks (preterm PDA); *other specified heart defect*; and *unspecified heart defect*. *Severe CHD* was defined as heterotaxia, conotruncal defect, AVSD, APVR, LVOTO, RVOTO (except valvular pulmonary stenosis), or other complex defect.

Information on chromosome aberrations and genetic conditions associated with CHD was retrieved using ICD codes [8th revision 759.3–759.5, 9th revision 758.0–759.9, 10th revision D82.1, Q87.1, Q87.2, Q90.0-Q99.9], van Mierop codes [8000–8004, 8009–8025, 8072], and by searching the free text fields for specific disorders (e.g. Down's syndrome, William's syndrome, Noonan's syndrome).

Folic acid and multivitamin supplementation

Since 1999, the Medical Birth Registry of Norway has registered maternal intake of periconceptional vitamins by using five check boxes on the Birth Registry notification form from maternity units; for regular use of folic acid or multivitamins before pregnancy or during pregnancy, or for no supplement use. The embryonic cardiac development is 3–7 weeks after conception, and includes the time period when the mother likely recognises her pregnancy, with subse-

© 2015 The Authors. Paediatric and Perinatal Epidemiology Published by John Wiley & Sons Ltd

Table 1. Maternal use of folic acid or multivitamin supplements before and during pregnancy in 517 784 births, Medical Birth Registry of Norway, 1999–2009[a]

		Supplement use during pregnancy			
		Folic acid		No folic acid	
Supplement use before pregnancy		Multivitamin No. (%)	No multivitamin No. (%)	Multivitamin No. (%)	No multivitamin No. (%)
Folic acid	Multivitamin	32 477 (6.3)	2196 (0.4)	1172 (0.2)	1538 (0.3)
	No multivitamin	16 485 (3.2)	36 725 (7.1)	1419 (0.3)	3497 (0.7)
No folic acid	Multivitamin	18 539 (3.6)	2572 (0.5)	12 496 (2.4)	1986 (0.4)
	No multivitamin	62 170 (12.0)	80 205 (15.5)	28 959 (5.6)	215 348 (41.6)

[a]Livebirths and stillbirths registered in the Medical Birth Registry with information on supplement use, excluding multiple births, births from in vitro fertilisation, children with chromosomal disorders, and with maternal epilepsy.

quent initiation of folic acid supplementation. We therefore decided to report risk estimates for CHD by any use of folic acid before pregnancy and/or during pregnancy, with or without multivitamin intake, referred to as periconceptional use of folic acid. Risk analyses were also performed for the other combinations of folic acid and multivitamin exposure (Table 1). Over-the-counter folic acid supplements in Norway contained 0.4 mg folic acid and multivitamin tablets 0.0–0.2 mg folic acid during the study period 1999–2009.

Other variables

We included the following covariates as confounders of the association between folic acid intake and infant CHD risk in the multivariable model; year of birth (each year), maternal age (<20, 20–24, 25–29, 30–34 and >34 years), parity (0, 1 or ≥2 previous pregnancies), maternal education (≤10, 11–13, 14–16, ≥17 years, missing (7.8%)), marital status (married/cohabitant, single/other), maternal smoking (regular/occasional, non-smokers, did not consent to register smoking information, missing (2.8%)), and family income (quartiles of mean income of the adults in the family). In initial analyses, we evaluated maternal diabetes (pregestational or gestational) as a confounder; however, diabetes did not affect the association between folic acid and CHD, and was not included in the final adjustment model.

Study population

In the period 1999–2009, 652 977 births were registered in the Medical Birth Registry of Norway. We excluded births with chromosomal disorders ($n = 2245$), multiple births ($n = 23\,815$), births from in vitro fertilisation ($n = 15\,791$), and births with maternal epilepsy ($n = 4978$), in total 41 292 (6.3%) births. Among the remaining 611 685 births, we excluded 93 901 (15.4%) births without information of folic acid or multivitamin supplementation (which included the 1442 pregnancies terminated for fetal reasons), leaving 517 784 livebirths and stillbirths for analyses.

Statistical analysis

The association between maternal periconceptional folic acid supplement use and infant risk of CHD was reported as relative risk (RR); the risk of CHD among the exposed divided by the risk of CHD among the unexposed. Births without maternal intake of folic acid or multivitamin supplements were used as reference group. Crude and adjusted relative risks (aRR) with 95% confidence intervals [CI] were estimated using binomial log linear regression models with a log-link function using STATA version 13 (Stata Corp., Texas, USA).

Results

Among 517 784 individuals, 6200 children had any type of CHD and 1153 had a severe CHD; the birth prevalence was 119.7 per 10 000 births and 22.3 per 10 000 births, respectively. In the period, the birth prevalence increased for total CHD until 2004, and decreased thereafter (Figure 1). The prevalence for severe CHD decreased from 2005. Overall, 95 509 (18.4%) mothers had initiated folic acid supplements

© 2015 The Authors. Paediatric and Perinatal Epidemiology Published by John Wiley & Sons Ltd

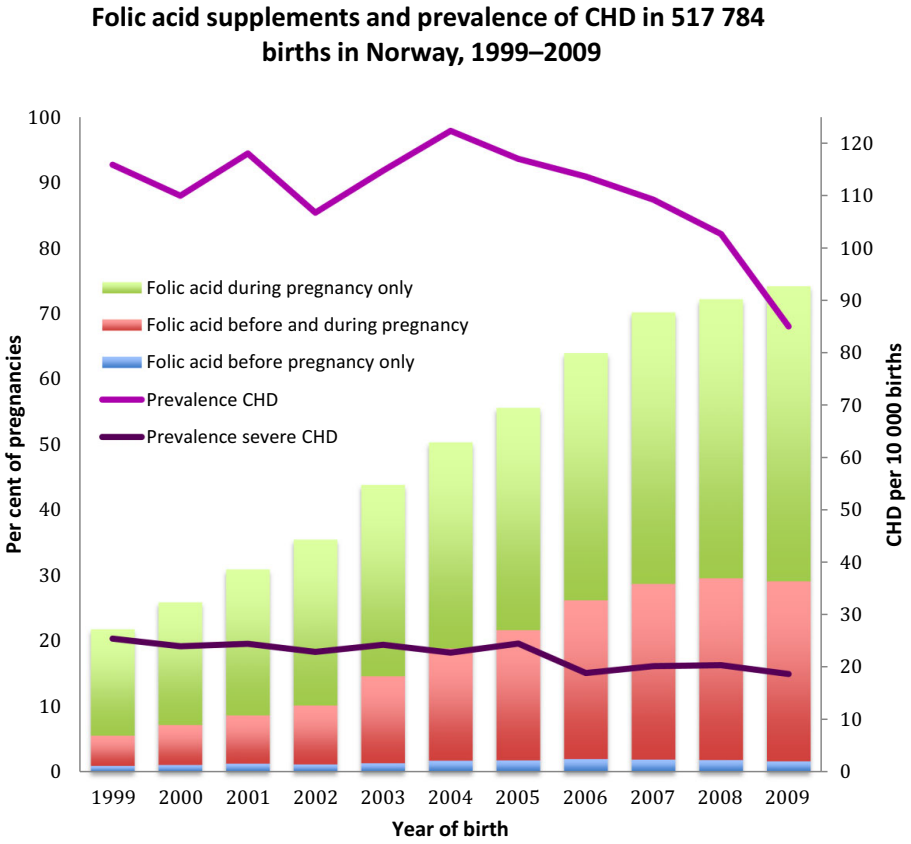


Figure 1. Periconceptional use of folic acid supplements and birth prevalence of severe CHD (heterotaxia, conotruncal defect, AVSD, APVR, LVOTO, RVOTO except valvular pulmonary stenosis, CoA, other complex heart defect) and any CHD except preterm PDA, by year of birth, in 517 784 livebirths and stillbirths registered in the Medical Birth Registry of Norway, 1999–2009, excluding multiple births, births from in vitro fertilisation, children with chromosomal disorders, and with maternal epilepsy. APVR, anomalous pulmonary venous return; AVSD, atrioventricular septal defect; CHD, congenital heart defect; CoA, coarctation of the aorta; LVOTO, left ventricle outflow tract obstruction; PDA, patent ductus arteriosus; RVOTO, right ventricle outflow tract obstruction.

before pregnancy, 163 486 (31.6%) had started during pregnancy, 43 441 (8.4%) used only multivitamins in the periconception period, while 215 348 (41.6%) did not use any folic acid or multivitamin supplements (Tables 1 and 2). Initiation of folic acid before pregnancy increased from 5.5% of all births in 1999 to 29.1% in 2009, while any periconceptional use of folic acid increased from 21.7% in 1999 to 74.2% in 2009.

In Table 2, initiation of folic acid supplements before pregnancy or during pregnancy, use of multivitamins only, and no use of any supplements are shown by year of birth and maternal characteristics. Shorter education, younger age, smoking, previous births, single status, and lower family income were more frequent in mothers who did not use any folic acid or multivitamin supplements.

Maternal periconceptional use of folic acid was not associated with infant risk of severe CHD; the aRR was 0.99 [95% CI 0.86, 1.13] comparing pregnancies exposed to folic acid with non-exposed pregnancies (Table 3). Specifically, aRR for conotruncal defects was 0.99 [95% CI 0.80, 1.22], aRR for AVSD 1.19 [95% CI 0.78, 1.81], aRR for LVOTO 1.02 [95% CI 0.78, 1.32],

© 2015 The Authors. Paediatric and Perinatal Epidemiology Published by John Wiley & Sons Ltd

Table 2. Birth characteristics according to use of folic acid before or during pregnancy in Norway, 1999–2009

Characteristics	Total births[a] 517 784		Preconceptional folic acid[b] 95 509 (18.4%)		Postconceptional folic acid only[c] 163 486 (31.6%)		Multivitamins only[d] 43 441 (8.4%)		No use of supplements[e] 215 348 (41.6%)	
	No.	% of Births	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Year of birth										
1999	46 520	(9.0)	2563	(5.5)	7558	(16.2)	5783	(12.4)	30 616	(65.8)
2000	48 472	(9.4)	3458	(7.1)	9080	(18.7)	4960	(10.2)	30 974	(63.9)
2001	46 693	(9.0)	4018	(8.6)	10 410	(22.3)	4532	(9.7)	27 733	(59.4)
2002	44 226	(8.5)	4475	(10.1)	11 202	(25.3)	4293	(9.7)	24 256	(54.8)
2003	43 749	(8.4)	6379	(14.6)	12 782	(29.2)	3900	(8.9)	20 688	(47.3)
2004	44 464	(8.6)	8376	(18.8)	13 992	(31.5)	3206	(7.2)	18 890	(42.5)
2005	44 614	(8.6)	9649	(21.6)	15 145	(33.9)	2957	(6.6)	16 863	(37.8)
2006	46 215	(8.9)	12 101	(26.2)	17 444	(37.7)	3523	(7.6)	13 147	(28.4)
2007	48 617	(9.4)	13 946	(28.7)	20 162	(41.5)	3661	(7.5)	10 848	(22.3)
2008	51 142	(9.9)	15 110	(29.5)	21 795	(42.6)	3386	(6.6)	10 851	(21.2)
2009	53 072	(10.2)	15 434	(29.1)	23 916	(45.1)	3240	(6.1)	10 482	(19.8)
Maternal education, y										
≤10	98 808	(19.1)	10 038	(10.2)	27 673	(28.0)	10 228	(10.4)	50 869	(51.5)
11–13	167 510	(32.4)	25 160	(15.0)	52 171	(31.1)	13 961	(8.3)	76 218	(45.5)
14–16	129 257	(25.0)	32 425	(25.1)	46 113	(35.7)	8317	(6.4)	42 402	(32.8)
≥17	81 713	(15.8)	23 618	(28.9)	27 933	(34.2)	4657	(5.7)	25 505	(31.2)
Missing data	40 496	(7.8)	4268	(10.5)	9596	(23.7)	6278	(15.5)	20 354	(50.3)
Maternal age, y										
<20	12 940	(2.5)	402	(3.1)	3475	(26.9)	1640	(12.7)	7423	(57.4)
20–24	79 284	(15.3)	7390	(9.3)	25 824	(32.6)	7940	(10.0)	38 130	(48.1)
25–29	172 574	(33.3)	31 139	(18.0)	56 181	(32.6)	14 050	(8.1)	71 204	(41.3)
30–34	169 622	(32.8)	38 081	(22.5)	52 764	(31.1)	12 798	(7.5)	65 979	(38.9)
>34	83 348	(16.1)	18 494	(22.2)	25 238	(30.3)	7010	(8.4)	32 606	(39.1)
Missing data	16	(0.0)	3	(18.8)	4	(25.0)	3	(18.8)	6	(37.5)
Maternal smoking										
No	357 552	(69.1)	75 196	(21.0)	115 748	(32.4)	28 762	(8.0)	137 846	(38.6)
Yes	94 429	(18.2)	9382	(9.9)	30 093	(31.9)	9777	(10.4)	45 177	(47.8)
No consent	59 007	(11.4)	9911	(16.8)	15 937	(27.0)	4420	(7.5)	28 739	(48.7)
Missing data	6796	(1.3)	1020	(15.0)	1708	(25.1)	482	(7.1)	3586	(52.8)
Parity										
0	209 839	(40.5)	39 570	(18.9)	75 789	(36.1)	17 871	(8.5)	76 609	(36.5)
1	186 530	(36.0)	38 025	(20.4)	56 236	(30.1)	14 290	(7.7)	77 979	(41.8)
≥2	121 415	(23.4)	17 914	(14.8)	31 461	(25.9)	11 280	(9.3)	60 760	(50.0)
Marital status										
Married/cohabiting	476 209	(92.0)	92 043	(19.3)	150 887	(31.7)	38 861	(8.2)	194 418	(40.8)
Single/other	41 575	(8.0)	3466	(8.3)	12 599	(30.3)	4580	(11.0)	20 930	(50.3)
Family income(quartiles)										
1	128 127	(24.7)	12 598	(9.8)	36 160	(28.2)	14 376	(11.2)	64 993	(50.7)
2	130 934	(25.3)	21 176	(16.2)	41 621	(31.8)	10 899	(8.3)	57 238	(43.7)
3	129 512	(25.0)	27 615	(21.3)	42 493	(32.8)	9393	(7.3)	50 011	(38.6)
4	124 504	(24.0)	33 706	(27.1)	42 472	(34.1)	8105	(6.5)	40 221	(32.3)
Missing data	4707	(0.9)	414	(8.8)	740	(15.7)	668	(14.2)	2885	(61.3)

[a]Livebirths and stillbirths registered in the Medical Birth Registry with information on supplement use, excluding multiple births, births from in vitro fertilisation, children with chromosomal disorders, and maternal epilepsy.
[b]Initiation of folic acid supplementation before pregnancy, with or without use of multivitamins.
[c]Initiation of folic acid supplementation during pregnancy, with or without use of multivitamins.
[d]Multivitamin supplements before and/or during pregnancy, with no use of folic acid.
[e]Pregnancies with no use of folic acid or multivitamin supplements as reference group.

© 2015 The Authors. Paediatric and Perinatal Epidemiology Published by John Wiley & Sons Ltd

Table 3. Congenital heart defects according to use of folic acid before and/or during pregnancy in 517 784 births in Norway, 1999–2009[a]

	Births	Any use of folic acid[b]		RR crude[c]		RR adjusted[d]	
Heart defect phenotype	No.	No.	%	RR	95% CI	aRR	95% CI
Any CHD	6200	3166	(51.1)	1.05	(0.99, 1.10)	1.10	(1.03, 1.16)
CHD excl. preterm PDA	5695	2928	(51.4)	1.07	(1.01, 1.13)	1.13	(1.06, 1.20)
Severe CHD	1153	546	(47.4)	0.89	(0.79, 1.01)	0.99	(0.86, 1.13)
Heterotaxia	71	28	(39.4)	0.69	(0.42, 1.13)	0.80	(0.45, 1.42)
Conotruncal defect	502	240	(47.8)	0.92	(0.77, 1.11)	0.99	(0.80, 1.22)
TGA	179	91	(50.8)	1.07	(0.78, 1.45)	1.10	(0.77, 1.57)
ToF	100	44	(44.0)	0.80	(0.53, 1.20)	0.90	(0.57, 1.44)
Other conotruncal[e]	223	105	(47.1)	0.87	(0.66, 1.15)	0.95	(0.70, 1.30)
AVSD	118	60	(50.8)	0.96	(0.66, 1.39)	1.19	(0.78, 1.81)
APVR	52	21	(40.4)	0.76	(0.42, 1.37)	0.78	(0.40, 1.52)
LVOTO	312	149	(47.8)	0.89	(0.70, 1.12)	1.02	(0.78, 1.32)
HLHS	78	40	(51.3)	0.98	(0.62, 1.55)	1.15	(0.69, 1.94)
CoA + IAA type A	109	53	(48.6)	0.92	(0.62, 1.36)	1.05	(0.67, 1.63)
vAS	125	56	(44.8)	0.80	(0.56, 1.16)	0.92	(0.61, 1.39)
RVOTO	258	120	(46.5)	0.89	(0.69, 1.15)	0.97	(0.72, 1.29)
HRHS	31	17	(54.8)	1.41	(0.65, 3.09)	1.43	(0.59, 3.42)
Ebstein	34	18	(52.9)	1.07	(0.53, 2.15)	0.91	(0.41, 1.99)
vPS	169	75	(44.4)	0.83	(0.60, 1.15)	0.96	(0.67, 1.38)
Other RVOTO[f]	24	10	(41.7)	0.64	(0.28, 1.46)	0.67	(0.26, 1.74)
Septal defect, isolated	3280	1747	(53.3)	1.16	(1.08, 1.25)	1.19	(1.10, 1.30)
ASD	647	345	(53.3)	1.16	(0.99, 1.37)	1.30	(1.08, 1.56)
VSD	2595	1378	(53.1)	1.15	(1.06, 1.25)	1.16	(1.06, 1.27)
Other septal defect	37	23	(62.2)	1.74	(0.85, 3.57)	1.97	(0.89, 4.38)
Other complex CHD[g]	9	3	(33.3)	0.50	(0.12, 2.09)	0.70	(0.15, 3.36)
Isolated PDA	1074	543	(50.6)	1.00	(0.88, 1.13)	1.02	(0.88, 1.17)
At term gestation	569	305	(53.6)	1.16	(0.98, 1.38)	1.23	(1.01, 1.50)
Preterm gestation	505	238	(47.1)	0.85	(0.71, 1.02)	0.82	(0.67, 1.01)
Other specified CHD	285	147	(51.6)	1.03	(0.81, 1.31)	1.17	(0.89, 1.53)
Unspecified CHD	239	108	(45.2)	0.80	(0.62, 1.04)	0.84	(0.62, 1.14)

[a]Livebirths and stillbirths registered in the Medical Birth Registry, excluding multiple births, births from in vitro fertilisation, children with chromosomal disorders, and with maternal epilepsy.
[b]Preconceptional or postconceptional initiation of folic acid, with or without use of multivitamins.
[c]Births with no periconceptional use of folic acid or multivitamin supplements as reference group.
[d]Relative risk adjusted for year of birth, parity, family income, mother's age, education, marital status, and smoking.
[e]Common arterial trunk, double outlet of the right ventricle (not ToF anatomy), conotruncal VSD, aortopulmonary window, supravalvular AS, IAA type B or C.
[f]Valvular or arterial pulmonary atresia.
[g]Single ventricle or ccTGAs.
APVR, anomalous pulmonary venous return; ASD, atrial septal defect; AVSD, atrioventricular septal defect; CHD, congenital heart defect; CoA, coarctation of the aorta; HLHS, hypoplastic left heart syndrome; HRHS, hypoplastic right heart syndrome; IAA, interrupted aortic arch; LVOTO, left ventricular outflow tract obstructions; PDA, patent ductus arteriosus; RVOTO, right ventricular outflow tract obstructions; TGA, transposition of the great arteries; ToF, tetralogy of Fallot; vPS, valvular pulmonary stenosis; VSD, ventricular septal defect.

and aRR for RVOTO 0.97 [95% CI 0.72, 1.29]. Septal defects (i.e. ASD, VSD, other septal defects) and term PDA were associated with increased risk in infants whose mothers used folic acid, as compared to non-users; aRRs were 1.19 [95% CI 1.10, 1.30] $P < 0.0001$ and 1.23 [95% CI 1.01, 1.50] $P = 0.04$, respectively.

The increased infant risk of septal defects among folic acid users were similar in categories of maternal education (≤10 years, aRR 1.26 [95% CI 1.05, 1.52]; 11–13 years, aRR 1.09 [95% CI 0.95, 1.26]; 14–16 years, aRR 1.36 [95% CI 1.15, 1.61]; ≥ 17 years, aRR 1.07 [95% CI 0.88, 1.30]), year of birth (1999–2002, aRR 1.24 [95%

© 2015 The Authors. Paediatric and Perinatal Epidemiology Published by John Wiley & Sons Ltd

Table 4. Congenital heart defects according to use of folic acid before and/or during pregnancy in 517 784 births in Norway, 1999–2009[a]

	Births	Preconceptional folic acid[b] (18.4%)		Postconceptional folic acid only[c] (31.6%)	
Heart defect phenotype	No.	aRR[d]	95% CI	aRR[d]	95% CI
CHD excl. preterm PDA	5695	1.14	(1.05, 1.24)	1.12	(1.04, 1.20)
Severe CHD	1153	1.10	(0.91, 1.33)	0.97	(0.83, 1.12)
Heterotaxia	71	1.14	(0.52, 2.49)	0.75	(0.39, 1.44)
Conotruncal defect	502	1.30	(0.99, 1.71)	0.90	(0.71, 1.14)
AVSD	118	1.07	(0.59, 1.96)	1.18	(0.75, 1.86)
APVR	52	0.70	(0.27, 1.83)	0.82	(0.42, 1.40)
LVOTO	312	0.93	(0.64, 1.36)	1.06	(0.80, 1.12)
RVOTO	258	0.98	(0.65, 1.47)	0.94	(0.68, 1.25)
Septal defect, isolated	3280	1.18	(1.06, 1.32)	1.19	(1.09, 1.31)
Other complex CHD	9	1.52	(0.24, 9.67)	0.44	(0.05, 3.90)
Isolated PDA at term GA	1074	1.16	(0.88, 1.52)	1.24	(1.00, 1.54)
Other specified CHD	285	1.39	(0.96, 2.01)	1.05	(0.77, 1.42)
Unspecified CHD	239	0.67	(0.43, 1.03)	0.91	(0.65, 1.27)

[a]Livebirths and stillbirths registered in the Medical Birth Registry, excluding multiple births, births from in vitro fertilisation, children with chromosomal disorders, and with maternal epilepsy.
[b]Initiation of folic acid supplementation before pregnancy, with or without use of multivitamins.
[c]Initiation of folic acid supplementation during pregnancy, with or without use of multivitamins.
[d]Relative risk adjusted for year of birth, parity, family income, mother's age, education, marital status and smoking. Births with no periconceptional use of folic acid or multivitamin supplements as reference group.
APVR, anomalous pulmonary venous return; AVSD, atrioventricular septal defect; CHD, congenital heart defect; GA, gestational age; LVOTO, left ventricular outflow tract obstructions; PDA, patent ductus arteriosus; RVOTO, right ventricular outflow tract obstructions.

CI 1.09, 1.42]; 2003–2006, aRR 1.19 [95% CI 1.05, 1.35]; 2007–2009, aRR 1.10 [95% CI 0.92, 1.32]), and gestational age (<37 weeks, aRR 1.16 [95% CI 0.94, 1.43]; ≥37 weeks, aRR 1.23 [95% CI 1.12, 1.34]).

In Table 4, the aRRs of the main CHD phenotypes are shown by initiation of folic acid supplementation before pregnancy (preconception) and during pregnancy (postconception). For preconceptional or postconceptional initiation of folic acid, the RRs were similar for severe defects (aRR 1.10 and aRR 0.97, respectively) and for septal defects (aRR 1.18 and aRR 1.19, respectively).

Finally, we estimated RR for CHD by various combinations of folic acid/multivitamin supplement exposures (folic acid only, folic acid and/or multivitamin supplement use before pregnancy, before and during pregnancy, or during pregnancy only) with similar results to those presented above (results not shown).

Comment

In this nationwide study of 517 784 births and 6200 children with CHD, 50% of the mothers had used folic acid supplementation in the periconceptional period. Maternal intake of folic acid supplements was not associated with infant risk of severe CHD, such as conotruncal defects, AVSD, LVOTO, and RVOTO. For septal defects, there was approximately 20% increased risk in children whose mothers had taken periconceptional folic acid supplements.

From mid-2000s there has been a decreasing prevalence of both severe and non-severe CHD in Norway, as in other European countries.[3,7] This downward time trend has been suggested explained by the increasing intake of periconceptional folic acid and multivitamins from 1999 to 2009.[10] In Quebec, Canada, the distinct decreasing prevalence of severe CHD from 1999 coincided with implementation of folic acid fortification of grain products from 1998.[6] In Alberta, Canada,[15] there was a reduction in LVOTO (mainly CoA) in the post folic acid fortification period, but no change in the overall CHD prevalence. There was no corresponding reduction in CHD in Atlanta, Georgia,[5] despite a similar food fortification policy. Such correlating time trends of folic acid supplement use or food fortification and the birth prevalence of CHD in

© 2015 The Authors. Paediatric and Perinatal Epidemiology Published by John Wiley & Sons Ltd

populations should be interpreted with caution, since the two events do not necessarily show a causal relationship.

Only a few studies have reported individual level information of maternal folic acid supplement use and infant risk of CHD. A Californian case-control study from 1995,[11] based on telephone interviews with the mothers of 207 children with conotruncal heart defects and 481 randomly selected infants without malformations, reported reduced risk for conotruncal heart defects in children of mothers who had taken multivitamins or folic acid fortified cereals. A registry-based case-control study from the Northern Netherlands 1996–2005,[13] including 611 children with CHD, and two control groups; 2401 supposedly non-folate-related birth defects, and 3343 pregnant women participating in previous cross-sectional studies, reported a reduced risk for CHD, mainly septal defects, in offspring of women using periconceptional folic acid supplements. In these studies, the folic acid content was similar to the presumed dose in our study (0.4 mg/d). In a case-control study from Hungary,[14] however, much higher doses were used; the estimated average dose was 5.6 mg/d. This Hungarian study compared 598 children with CHD born in 1980–1996 with 902 matched controls, 20 896 children with other malformations, and 38 151 children without birth defects, and reported a reduced risk for conotruncal heart defect in the group exposed for folic acid supplements. In a Hungarian randomised controlled trial,[12] multivitamin supplements with 0.8 mg folic acid were compared to supplements with other trace elements. There was significantly reduced risk for CHD in the group receiving vitamins with high-dose folic acid, but the numbers were small, with only 10 vs. 20 CHD cases in the exposed and unexposed group, respectively.

A possible risk reduction of CHD by intake of folic acid or multivitamin supplements is likely determined by the extent of dietary vitamin insufficiency in the population. The dietary folate intake reported in pregnant Norwegian women, around 300 µg per day,[21] could be sufficient for fetal cardiac development, as opposed to certain Chinese provinces with a high prevalence of folate deficiency, where the risk of CHD has been significantly reduced when mothers had used periconceptional folic acid supplements.[22] Alternatively, the dose of folic acid supplement in the present study, 400 µg per day, is too small to prevent cardiac malformations, although plasma folate has been found to be significantly higher in women reporting folic acid supplement use.[23] However, no risk reduction was found, even in the group with both folic acid and multivitamin supplements before and during pregnancy (approximately folic acid dose of 0.6 mg/d), suggesting the use of folic acid supplements does not prevent CHD in the Norwegian population. Changes in other risk factors may explain the recent decrease in CHD prevalence.

To our surprise, we found a significantly increased risk for both ASD and VSD if the mother had used folic acid supplements in the periconceptional period, which has not been reported previously. The increased risk of septal defects was not modified by year of birth, maternal education, or prematurity. The positive association between folic acid supplements and risk of septal defects in the present study is unlikely a chance finding but could be caused by an unknown residual confounding. A biological factor cannot be ruled out; in studies of pregnant mice, a moderate to high intake of folic acid had adverse effects on offspring cardiac development. Mikael *et al*. found that mice fed with a diet containing 10 times the recommended rodent folic acid intake showed an increased risk for VSDs and thinner ventricular walls than the control group.[24]

The strengths of our nationwide study were the cohort design, linkage of comprehensive and compulsory registries with reliable information, and minimal loss to follow-up. Ascertainment of CHD cases through four national administrative and clinical registries enabled a virtually complete registration of both severe and minor CHD.[3] A weakness may be related to the validity of the exposure variable, maternal intake of folic acid or multivitamins, as we had no information on dose or duration of intake. However, the only folic acid tablets sold in Norway during the study period contained the recommended daily dose of 0.4 mg folic acid, and the maximum folic acid content in multivitamins was 0.2 mg. Although a large proportion of women started folic acid supplementation during pregnancy in the present study, we know from another Norwegian study in the same period that almost all folic acid users had implemented supplementation at 4 to 5 gestational week, corresponding to 2 to 3 weeks post conception (85 000 pregnancies in the Norwegian Mother and Child Cohort Study, 2001–2008),[25] which should cover most of the heart development period ranging from 3 to 7

© 2015 The Authors. Paediatric and Perinatal Epidemiology Published by John Wiley & Sons Ltd

weeks post conception. All combinations of folic acid or multivitamin intake before pregnancy or during pregnancy showed consistent findings; the association between folic acid or multivitamin supplement use and severe CHD was null, and for septal defects, there was a slightly increased risk after folic acid supplement use. Other concerns might be lack of details on additional confounders of the association between maternal supplementation and maternal/offspring outcome, such as other dietary nutrients, or maternal pre-pregnancy weight. We did not have such information. Information on supplement use was missing in 15% of all births; these births were excluded from the study population. However, the prevalence of severe defects and septal defects were similar among births with missing folic acid information and the study population, except for the terminated pregnancies (0.2% of all births). The prevalence of CHD was increased in all terminated pregnancies (144 per 1000), but the proportion of terminated pregnancies among all births with any CHD (2.7%) or severe CHD (9.3%) was low, and amounted to a relatively small part of the births with CHD.

In conclusion, periconceptional use of folic acid or multivitamin supplements was not associated with risk of severe CHDs in infants. The association with a 20% increased risk of septal defects may be due to unknown common factors for folic acid use and CHD risk, or an adverse effect from folic acid, and warrants further investigation.

Acknowledgements

The authors thank Tomislav Dimoski at the Norwegian Knowledge Centre for the Health services, Oslo, Norway, for his contribution by developing software necessary for obtaining data from Norwegian hospitals, conducting the data collection and quality assurance of data in this project. The study was funded by Research Council Norway, project number 190858/V50 to Dr. Nina Øyen.

Disclosures

The authors are solely responsible for the design and conduct of this study, all study analyses, and the drafting and editing of the manuscript and its final contents.

References

1 Hoffman JI, Kaplan S. The incidence of congenital heart disease. *Journal of the American College of Cardiology* 2002; 39:1890–1900.
2 Botto LD, Lin AE, Riehle-Colarusso T, Malik S, Correa A. Seeking causes: classifying and evaluating congenital heart defects in etiologic studies. *Birth Defects Research. Part A, Clinical and Molecular Teratology* 2007; 79:714–727.
3 Leirgul E, Fomina T, Brodwall K, Greve G, Holmstrom H, Vollset SE, *et al*. Birth prevalence of congenital heart defects in Norway 1994–2009 – a nationwide study. *American Heart Journal* 2014; 168:956–964.
4 Øyen N, Poulsen G, Boyd HA, Wohlfahrt J, Jensen PK, Melbye M. National time trends in congenital heart defects, Denmark, 1977–2005. *American Heart Journal* 2009; 157:467–473 e461.
5 Bjornard K, Riehle-Colarusso T, Gilboa SM, Correa A. Patterns in the prevalence of congenital heart defects, metropolitan Atlanta, 1978 to 2005. *Birth Defects Research. Part A, Clinical and Molecular Teratology* 2013; 97:87–94.
6 Ionescu-Ittu R, Marelli AJ, Mackie AS, Pilote L. Prevalence of severe congenital heart disease after folic acid fortification of grain products: time trend analysis in Quebec, Canada. *BMJ (Clinical Research Ed.)* 2009; 338:b1673.
7 Khoshnood B, Loane M, Garne E, Addor MC, Arriola L, Bakker M, *et al*. Recent decrease in the prevalence of congenital heart defects in Europe. *Journal of Pediatrics* 2013; 162:108–113 e102.
8 Milunsky A, Jick H, Jick SS, Bruell CL, MacLaughlin DS, Rothman KJ, *et al*. Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. *JAMA: The Journal of the American Medical Association* 1989; 262:2847–2852.
9 Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *New England Journal of Medicine* 1992; 327:1832–1835.
10 Daltveit AK, Vollset SE, Lande B, Øien H. Changes in knowledge and attitudes of folate, and use of dietary supplements among women of reproductive age in Norway 1998–2000. *Scandinavian Journal of Public Health* 2004; 32:264–271.
11 Shaw GM, O'Malley CD, Wasserman CR, Tolarova MM, Lammer EJ. Maternal periconceptional use of multivitamins and reduced risk for conotruncal heart defects and limb deficiencies among offspring. *American Journal of Medical Genetics* 1995; 59:536–545.
12 Czeizel AE. Periconceptional folic acid containing multivitamin supplementation. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* 1998; 78:151–161.
13 van Beynum IM, Kapusta L, Bakker MK, den Heijer M, Blom HJ, de Walle HE. Protective effect of periconceptional folic acid supplements on the risk of congenital heart defects: a registry-based case-control study in the northern Netherlands. *European Heart Journal* 2010; 31:464–471.
14 Csaky-Szunyogh M, Vereczkey A, Kosa Z, Gerencser B, Czeizel AE. Risk and protective factors in the origin of conotruncal defects of heart – a population-based

© 2015 The Authors. Paediatric and Perinatal Epidemiology Published by John Wiley & Sons Ltd

case-control study. *American Journal of Medical Genetics. Part A* 2013; 161A:2444–2452.
15 Bedard T, Lowry RB, Sibbald B, Harder JR, Trevenen C, Horobec V, *et al*. Folic acid fortification and the birth prevalence of congenital heart defect cases in Alberta, Canada. *Birth Defects Research. Part A, Clinical and Molecular Teratology* 2013; 97:564–570.
16 Botto LD, Lisi A, Bower C, Canfield MA, Dattani N, De Vigan C, *et al*. Trends of selected malformations in relation to folic acid recommendations and fortification: an international assessment. *Birth Defects Research. Part A, Clinical and Molecular Teratology* 2006; 76:693–705.
17 Irgens LM. The Medical Birth Registry of Norway. Epidemiological research and surveillance throughout 30 years. *Acta Obstetricia et Gynecologica Scandinavica* 2000; 79:435–439.
18 Hagemo PS. BERTE – a database for children with congenital heart defects. *Studies in Health Technology and Informatics* 1994; 14:98–101.
19 Sulo GIJ, Vollset SE, Nygård O, Øyen N, Tell GS. Cardiovascular disease and diabetes mellitus in Norway during 1994–2009 CVDNOR – a nationwide research project. *Norwegian journal of epidemiology.* 2013; 23:101–107.
20 Statistics Norway. Available from http://www.ssb.no/en/forside. [last accessed 20 July 2015].
21 Sengpiel V, Bacelis J, Myhre R, Myking S, Devold Pay A, Haugen M, *et al*. Folic acid supplementation, dietary folate intake during pregnancy and risk for spontaneous preterm delivery: a prospective observational cohort study. *BMC Pregnancy and Childbirth* 2014; 14:375.
22 Li X, Li S, Mu D, Liu Z, Li Y, Lin Y, *et al*. The association between periconceptional folic acid supplementation and congenital heart defects: a case-control study in China. *Preventive Medicine* 2013; 56:385–389.
23 Roth C, Bjørke-Monsen AL, Reichborn-Kjennerud T, Nilsen RM, Smith GD, Stoltenberg C, *et al*. Use of folic acid supplements in early pregnancy in relation to maternal plasma levels in week 18 of pregnancy. *Molecular Nutrition & Food Research* 2013; 57:653–660.
24 Mikael LG, Deng L, Paul L, Selhub J, Rozen R. Moderately high intake of folic acid has a negative impact on mouse embryonic development. *Birth Defects Research. Part A, Clinical and Molecular Teratology* 2013; 97:47–52.
25 Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, *et al*. Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. *JAMA: The Journal of the American Medical Association* 2013; 309:570–577.

© 2015 The Authors. Paediatric and Perinatal Epidemiology Published by John Wiley & Sons Ltd