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PRENATAL RISK FACTORS FOR SEVERE CARDIOVASCULAR DISEASES UP TO MIDDLE-AGE: A NORDIC COLLABORATIVE STUDY

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Stockholm 2024

Prenatal risk factors for severe cardiovascular diseases up to middle-age: a Nordic collaborative study
Thesis for Doctoral Degree (Ph.D.)

By

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The thesis will be defended in public in the lecture room David, Widerströmska building, Tomtebodavägen 18A, Solna and online via Zoom
February 16th 2024 at 9:00 AM

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To my Papa and Laolao,

who inspired my pursuit of "why."

ABSTRACT

Background and objectives

Cardiovascular diseases (CVDs) are major causes of death and disability. However, the established traditional risk factors cannot explain a substantial proportion of CVD cases, prompting investigations into novel risk factors. A growing body of evidence underscores the potential role of suboptimal intrauterine conditions on the development of CVD. Nonetheless, our knowledge about the associations between factors contributing to an adverse intrauterine environment and the risk of developing CVD remains limited. The overall objective of this thesis is to enhance our comprehension of the potential role of prenatal risk factors in developing CVD later in life. More specifically, the thesis aims to study the following research questions: (1) Are negative birth outcomes such as preterm birth, being small (SGA) or large (LGA) for gestational age related to the atrial fibrillation risk later in life (Study I)? (2) Is maternal preeclampsia and its subtypes linked to increased risks of stroke and ischemic heart disease in the offspring (Study II)? (3) Is maternal polycystic ovary syndrome (PCOS) associated with the risks of overall CVD and its major subtypes in her offspring (Study III)? and (4) Is prenatal exposure to maternal severe stress related to the risk of heart failure later in life (Study IV)?

Methods

We performed four register-based prospective cohort studies, including all live singletons from Denmark (Study I: 1978-2016, Studies II-IV: 1973-2016) and Sweden (Studies I-IV: 1973-2014), and live births from a randomly selected 90% of all births in Finland Studies I and II; 1987-2014). The size of the study population was 8,012,433 in Study I, 8,475,819 in Study II, 6,839,703 in Study III, and 6,758,560 in Study IV. Information on birth outcomes, maternal and offspring's health and covariates were obtained through linkage to population-based socioeconomic and health registers. Each study participant was followed up until the earliest diagnosis of the CVD of interest, emigration, death, or end of follow-up (Denmark: December 31, 2016; Finland: December 31, 2014; Sweden: December 31, 2020), whichever occurred first. We examined the association between prenatal exposures (including preterm birth, SGA, LGA, maternal preeclampsia, PCOS, and severe stress) and CVD outcomes in offspring using multivariable Cox regression models. Furthermore, we used family-based study designs, i.e. sibling and cousin comparison analyses, to account for unmeasured familial genetic and environmental confounders. Additionally, we investigated the mediating roles of abnormal birth outcomes and congenital heart disease in case of some of the observed associations.

Results

In Study I, we found that being born preterm or LGA was linked to an increased risk of atrial fibrillation in both childhood and adulthood. The associations persisted in the sibling comparison analyses. In contrast, SGA was related to an increased atrial fibrillation risk in childhood but not in adulthood.

In Study II, we found that individuals prenatally exposed to maternal preeclampsia had higher risks of stroke and ischemic heart disease than those unexposed, and that the associations were more pronounced in cases of severe than milder forms of preeclampsia. The associations of the severe forms of maternal preeclampsia with the offspring's risk of stroke remained in the sibling comparison analyses.

In Study III, maternal PCOS was associated with elevated risks of overall CVD, hypertensive disease, stroke, and ischemic heart disease in the population analysis; most of these associations, except that observed in case of stroke, remained in the cousin comparison analysis. When investigating the interaction between maternal PCOS and its prevalent comorbidities, we found that individuals born to mothers with both PCOS and its common comorbid conditions, i.e. diabetes, hypertensive disease, or psychiatric disorders, had higher CVD risks than those born to mothers with only PCOS.

In Study IV, we found that offspring exposed to maternal loss of a close family member the year prior to or during pregnancy did not have a higher risk of heart failure than those unexposed. However, the severe forms of maternal bereavement, specifically loss due to unnatural causes and loss of a child or partner, were linked to an increased risk of heart failure in the offspring.

When splitting follow-up for Studies I-IV at the age of 18, we found that the association between preterm birth and the risk of atrial fibrillation, maternal preeclampsia and the risk of stroke, and severe maternal stress and the risk of heart failure were stronger during childhood than during adulthood.

In the mediation analyses, there was some evidence that the association between maternal PCOS and the risk of CVD in offspring was to a modest extent mediated by preterm birth, LGA, and congenital heart disease. In the case of the link between severe maternal stress and the risk of heart failure, we observed considerable contributions from congenital heart disease and preterm birth.

Conclusions

This thesis revealed associations of prenatal risk factors with increased risks of CVD up to early middle-age. Specifically, our findings suggest that preterm birth, SGA, and LGA were related to elevated risks of atrial fibrillation. Additionally, maternal preeclampsia, especially its severe types, was associated with elevated risks of stroke and ischemic heart disease in offspring, while maternal PCOS was associated with increased risks of overall CVD and its major types. Moreover, severe maternal stress was associated with an elevated risk of heart failure in offspring. If subsequent studies confirm our findings, early-life prevention and targeted intervention programs may be developed to inform health policies, eventually resulting in a reduced burden of CVD.

LIST OF SCIENTIFIC PAPERS

I. **Yang F**, Janszky I, Gissler M, Cnattingius S, Roos N, Miao MH, Yuan W, Li J, László KD. Preterm birth, small for gestational age, and large for gestational age and the risk of atrial fibrillation up to middle age. JAMA Pediatrics. 2023; 177(6): 599-607.

II. **Yang F**, Janszky I, Gissler M, Roos N, Wikström AK, Yu YF, Chen H, Bonamy AKE, Li J, László KD. Association of maternal preeclampsia with offspring risks of ischemic heart disease and stroke in Nordic countries. JAMA Network Open. 2022; 5(11): e2242064.

III. **Yang F**, Wang ZL, Sørensen HT, Janszky I, Gissler M, Yuan W, Miao MH, Roos N, Wikström AK, Li J, László KD. Risk of cardiovascular diseases in offspring of women with polycystic ovary syndrome: a binational cohort study [Submitted].

IV. **Yang F**, Janszky I, Roos N, Li J, László KD. Severe maternal stress during pregnancy and the offspring's risk of heart failure in the first five decades of life: a binational cohort study [Accepted in JACC: Heart Failure].

SCIENTIFIC PAPERS NOT INCLUDED IN THE THESIS

I. Liu QW, László KD, Wei D, **Yang F**, Fall K, Valdimarsdóttir U, Feychting M, Li J, Fang F. Suicide attempt and death by suicide among parents of young individuals with cancer: a population-based study in Denmark and Sweden [Accepted in PLOS Medicine].

II. **Yang F**, Janszky I, Roos N, Li J, László KD. Prenatal exposure to severe stress and risks of ischemic heart disease and stroke in offspring. JAMA Network Open. 2023; 6(12): e2349463.

III. Ji HL, Guo M, **Yang F**, Liang H, Wang ZL, Chen Y, Zheng HJ, Miao MH, Yuan W. Prenatal per- and polyfluoroalkyl substances exposure and gut microbiota of infants: A prospective cohort study. Ecotoxicology and Environmental Safety. 2024; 270: 115891.

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V. Luan M, **Yang F**, Miao MH, Yuan W, Gissler M, Arkema EV, Lu DH, Li J, László KD. Rheumatoid arthritis and the risk of postpartum psychiatric disorders: a Nordic population-based cohort study. BMC Medicine. 2023; 21(1):126.

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VII. Hu KJ, Liu QW, László KD, Wei D, **Yang F**, Fall K, Adami HO, Ye WM, Valdimarsdóttir U, Li J, Fang F. Risk of psychiatric disorders among spouses of patients with cancer in Denmark and Sweden. JAMA Network Open. 2023; 6(1): e2249560.

VIII. Li F*, **Yang F***, Li DK, Tian YP, Miao MH, Zhang Y, Ji HL, Yuan W, Liang H. Prenatal bisphenol A exposure, fetal thyroid hormones and neurobehavioral development in children at 2 and 4 years: A prospective cohort study. Science of The Total Environment. 2022; 722:137887.

IX. Luan M, Zhang XH, Fang GH, Liang H, **Yang F**, Song XX, Chen Y, Yuan W, Miao MH. Preconceptional paternal alcohol consumption and the risk of child behavioral problems: a prospective cohort study. Scientific Reports. 2022; 12(1): 1-11.

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Contents

LIST OF ABBREVIATIONS

AF	Atrial fibrillation
AGA	Appropriate for gestational age
BMI	Body-mass index
CI	Confidence intervals
CVD	Cardiovascular disease
DOHaD	Developmental origins of health and disease
HBW	High birth weight
HDP	Hypertensive disorders during pregnancy
HF	Heart failure
HPA	Hypothalamus-pituitary-adrenocortical
HR	Hazard ratio
ICD	International Classification of Diseases
IHD	Ischemic heart disease
LBW	Low birth weight
LGA	Large for gestational age
PCOS	Polycystic ovary syndrome
RAAS	Renin-angiotensin-aldosterone system
SGA	Small for gestational age

1 INTRODUCTION

Cardiovascular diseases (CVD) are significant contributors to mortality and morbidity globally.[1] Over the last four decades, developed countries have experienced a significant decline in age-adjusted CVD rates. This is attributed mainly to the identification of major risk factors for CVD, advancements in primary and secondary prevention, and the development and introduction of effective therapies for ischemic heart disease (IHD) and stroke, which are the two major types of CVD. With improvements in the survival of patients with IHD and stroke, the incidence of other severe CVDs, such as heart failure (HF) and atrial fibrillation (AF), has been rising. The well-established environmental, lifestyle, and genetic risk factors cannot explain a substantial proportion of CVD cases.[2-5] Consequently, it is necessary to explore and identify further novel risk factors for CVD to inform prevention efforts.

According to 'developmental origins of adult disease hypothesis,' adverse conditions in utero may program the foetus' endocrine and metabolic activity, and may potentially be involved in the development of CVD after birth.[6] Increasing evidence suggests that unfavorable birth outcomes, such as low birth weight and preterm birth, may increase the risk of several CVDs, including hypertension, IHD, stroke and HF.[7-12] However, knowledge regarding their associations with AF is very limited. Preterm birth and abnormal foetal growth are, in turn, associated with several maternal diseases during pregnancy, such as preeclampsia or polycystic ovary syndrome (PCOS), as well as with severe maternal stress during pregnancy. The prevalence of maternal chronic diseases during pregnancy has risen in recent decades in Western countries.[13] This trend is attributed to delayed motherhood, a general increase in non-communicable diseases in the total population, and improved medical care enabling women with severe illnesses, who once had to refrain from childbirth, to now plan pregnancies. Accumulating evidence indicates that a mother's diseases or severe stress during pregnancy may affect gestational age and foetal growth, and long-term health related to immune, neuropsychiatric, metabolic, and reproductive systems, which may further increase the risk of CVD later in life.[1,14,15] Consequently, a link between these maternal conditions during pregnancy and an increased risk of CVD in the offspring is plausible. However, empirical evidence in this area is limited and inconsistent. Additionally, it remains uncertain to what extent such eventual associations may be explained by genetic and environmental risk factors shared between mother and child.

This thesis aims to explore the role of prenatal risk factors, namely preterm birth, abnormal foetal growth, maternal preeclampsia, polycystic ovary syndrome, and severe stress, in the development of CVD later in life. Through a registry-based cohort study including Danish, Swedish, and Finnish live singleton births, we investigated associations between preterm birth, small and large for gestational age, and the risk of AF (Study I); maternal preeclampsia and its subtypes and offspring's risks of IHD and stroke (Study II); maternal PCOS and offspring's risks of overall CVD and major CVD types (Study III); as well as severe maternal stress, i.e., bereavement the year before or during pregnancy, and offspring's risk of HF later in life (Study IV).

2 LITERATURE REVIEW

2.1 Epidemiology of cardiovascular diseases

As the leading cause of mortality and disability, cardiovascular diseases (CVDs) contribute to approximately one-third of deaths globally.[1] The global number of CVD-related deaths was 12.1 million in 1990 and increased to 18.6 million in 2019. It is projected that this number will increase further, to 23 million by 2030,[16] driven mainly by population growth and aging.[17] The age-adjusted prevalence of CVD has decreased considerably in most developed countries since the early 1970s. This trend is attributed primarily to the identification of major CVD risk factors and effective prevention and therapy; however, the decline has slowed in several countries.[18,19]

Similarly, in Europe, CVD ranks as the main cause of death, causing annually more than 4 million deaths, i.e. 45% of all deaths.[20] Although their age-specific incidence rates have decreased consistently between 1990 and 2019, death due to ischemic heart disease (IHD) and stroke remain the most common, representing approximately 50% and 35%, respectively of all CVD deaths.[21] Furthermore, atrial fibrillation (AF), the most prevalent cardiac arrhythmia, has emerged as a significant public health concern in recent years. In contrast to other CVDs, the age-adjusted prevalence and incidence of AF show a modestly increasing trend.[22] The prevalence of AF in Europe has doubled in the last decades to 2% and is expected to rise to 2.7-3.3% by 2030.[23] Another CVD warranting special attention is heart failure (HF), which is often the end stage of other heart diseases and which is associated with high mortality.[24] According to the latest available statistics, more than15 million individuals in Europe live with HF, and its prevalence continues to increase due to advancements in diagnosis and treatment.[25]

Although the major CVDs such as IHD, stroke, AF, or HF mainly affect the elderly, over the past three decades there has been a stable or a slightly increasing trend in the incidence rates of these conditions in young adults.[26,27] For example, a Danish nationwide study showed that the incidence of HF among individuals younger than 50 years was 1.02 per 10,000 person-years in 1995 and increased to 1.52 per 10,000 person-years in 2012, while the incidence among individuals 50 years or older declined during the same period from 1.22 in 1995 to 0.62 in 2012 per 10,000 person-years, respectively.[28] A comparable trend for HF in young individuals was also observed in Sweden.[29] A young patient with CVD imposes a more important socio-economic burden than an older CVD patient, primarily because of productivity loss at an early age and the extended duration of healthcare needs.

Given the substantial CVD burden, research regarding mechanisms and risk factors involved in the development of CVD is crucial for the effective prevention of CVD. Until relatively recently, most studies concerning aetiology focused on behavioural, physiological, and psychosocial risk factors such as unhealthy diet, low physical activity, tobacco use, dyslipidaemia, obesity, hypertension, diabetes mellitus, gender, and stress.[30] However, there is a proportion of patients who are not affected by the above risk factors and still suffer from CVD.[2-5] Besides, the aetiology and risk factors for CVD in children and young adults could differ from those in older patients. However, research on this topic is sparse due to difficulties in conducting sufficiently large studies with young patients. It has been increasingly recognized that factors influencing the risk of CVD could act early in life, even in the foetal period. In the following sections of this review, the thesis will focus on the potential role of

prenatal risk factors in the development of CVD, especially some important CVDs such as IHD, stroke, AF, and HF.

2.2　Foetal programming of CVD

The 'developmental origins of adult disease hypothesis' (also referred to as the 'foetal origins hypothesis' or 'Barker hypothesis') [31] posits that adverse conditions early in life, particularly during intrauterine life, can program the offspring's neuroendocrine, physiological, and metabolic activities. This programming may increase the risk of chronic diseases in adulthood, including CVD, diabetes, obesity or cancer.[32] The idea that environmental conditions in early life are associated with mortality in adulthood was first proposed by Kermack in 1934.[33] Forsdahl stimulated the research interest in this hypothesis in the early 1970s by showing an association between adverse living conditions in early life and a heightened risk of heart disease in Norway and Finland.[34,35] Forsdahl's findings were replicated in different countries and led to the formulation of the 'foetal origins hypothesis' by David Barker and colleagues, evolving into the broader theory of 'developmental origins of health and disease (DOHaD).' Barker postulated that adverse influences, such as foetal undernutrition, leading to impaired foetal growth, could increase the risks of hypertension, IHD, and stroke in adulthood.[36] While initial explorations related to the DOHaD theory focused on foetal undernutrition and subsequent low birth weight, numerous researchers have since expanded the scope of the theory. Multiple perinatal risk factors, including overnutrition, preterm delivery, maternal pregnancy complications, other medical conditions or stress during pregnancy, are now recognized as potential *in-utero* insults contributing to the long-term health outcomes outlined in the DOHaD hypothesis.[37]

2.3　Adverse birth outcomes and the risk of CVD

2.3.1　Birth weight

Low birth weight (LBW, defined as birth weight below 2.5 kilograms) serves as a surrogate marker for an adverse intrauterine environment. Foetal malnutrition, a potential consequence of placental dysfunction, can lead to LBW. Most studies on cardiovascular risk related to birth weight have focused on LBW. While some studies in this field have reported null findings,[38,39] a substantial body of observational research across diverse settings consistently indicates that individuals born with LBW are at elevated risk of developing CVD in adulthood, including IHD,[11,40-55] stroke,[11,44,55-57] AF,[58] and HF.[59] The findings from these studies have been synthesized in three meta-analyses, revealing that a one-kilogram increase in birth weight is linked to a 10–20% reduction in the risk of developing CVD.[15,60,61]

High birth weight (HBW, defined as birth weight exceeding 4 or 4.5 kilograms) may be a consequence of maternal factors such as gestational diabetes mellitus, obesity, or excessive weight gain during pregnancy.[62] Individuals born with HBW are at increased risk of developing obesity and diabetes,[63-65] both known risk factors for CVD. While only a few studies have established an link between HBW and CVD,[66,67] this potential link is noteworthy.

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A linear model may thus not adequately capture the relationship between birth weight and CVD. Some studies have shown a non-linear association ('J'- or 'U'-shaped) of birth weight with overall CVD,[68,69] IHD,[51,69] stroke,[69] and AF,[70,71] with increased risk among both those in the lowest and the highest birthweight categories. A recent meta-analysis of 24 observational studies[72] found that the risk of CVD increased as birth weight decreased below 2.5 kilograms and increased above 4 kilograms. Findings from a recent cohort study using data from the UK Biobank suggested that individuals with a birth weight ranging from 3.41 to 3.79 kilograms had the lowest CVD risk.[69]

The mixed results observed in earlier studies connecting birth weight to CVD risk could be ascribed to diverse classifications of birth weight, the selection of reference groups, limited sample sizes, or inadequate follow-up periods. Furthermore, some studies may have been susceptible to recall bias, given that birth weight was frequently self-reported. Additionally, several studies did not adjust for important confounders including maternal smoking, gestational diabetes, hypertensive disorders, and genetic predisposition. Moreover, the strong correlation between birth weight and gestational age raises the question of the extent to which birth weight alone plays a role in the observed associations.

2.3.2   Abnormal foetal growth

Birth weight is a composite outcome influenced by both foetal growth and gestational age. However, there have been limited attempts in existing studies to disentangle the distinct contributions of these two factors to the relationship between birth weight and CVD risk. The Uppsala Birth Cohort, consisting of 15,000 individuals born between 1915 and 1929, was among the first to provide evidence that mortality from IHD was linked to foetal growth (measured as birth weight for gestational age) rather than gestational age.[44] Similarly, another birth cohort study including all births at four central delivery units in Sweden from 1925 to 1949[48] suggested that the association between LBW and the risk of IHD might stem from small for gestational age (SGA, a term commonly used to describe restricted foetal growth) rather than preterm birth (before 37 weeks of gestation). These findings were consistent with historical cohort studies that have documented associations between foetal growth restriction and the risks of IHD, acute myocardial infarction, and stroke.[52-54,73,74] However, similarly to the 'U'-shaped relationship found between birth weight and CVD, the association of foetal growth with CVD may also be non-linear. Currently, there is increasing attention on excessive foetal growth, which has been reported to be associated with cardiometabolic disorders in both childhood and adulthood.[75,76] Nevertheless, there is a lack of studies exploring the association between being large for gestational age (LGA, a proxy for excessive foetal growth) and the risk of CVD later in life.

2.3.3   Preterm birth

Preterm birth affects approximately 11% of live births globally[77] and 5-8% of births in Europe.[78] Recent findings by Crump and colleagues, derived from a Swedish national cohort study with a follow-up up to 43 years, document that preterm birth is linked to increased risks of hypertension,[7] IHD,[8] stroke,[9] HF,[10] and overall CVD mortality,[79] irrespective of foetal growth. In contrast, two Swedish birth cohort studies[44,48] and the Helsinki Birth Cohort Study[74] found no association between gestational age at birth and IHD or stroke risk. Only two historical cohorts investigated whether

preterm birth was associated with the risk of AF, and none of them observed an association.[70,71] Discrepancies among the studies may be attributed to survivor bias, as participants in the Swedish or Helsinki birth cohorts were born before the 1950s when modern neonatal care was not available. Consequently, those in these historical cohorts who survived after preterm birth may be healthier and less susceptible to CVD compared to contemporary preterm birth survivors in Crump's study.

There are several possible explanations for the increased risk of CVD among individuals born prematurely or with abnormal foetal growth. Primarily, *in-utero* insults such as malnutrition or overnutrition, reflected through abnormal foetal growth and gestation age, could trigger adaptations in the renin angiotensin aldosterone system (RAAS), hypothalamus-pituitary-adrenocortical (HPA) axis, and sympathetic nervous system, ultimately leading to physiological alterations such as insulin resistance and cardiovascular remodelling and dysfunction.[37] These alterations, in turn, increase the likelihood of developing metabolic disease, hypertension, IHD, stroke, and other forms of CVD later in life. An additional plausible mechanism is that the *in-utero* insults might result in genetic alterations via epigenetic processes. Specific epigenetic changes that may contribute to the progress of CVD include angiotensin-converting enzyme, 11 beta-hydroxysteroid dehydrogenase type 2, and angiotensin type 2 receptor.[37]

Focusing solely on the associations between adverse foetal growth and CVD risk later in life might not fully elucidate the complete causal pathway for CVD development since foetal growth is a result of the interaction between the intrauterine environment and genetic make-up. The intrauterine environment, in turn, is determined by maternal factors (including nutritional status, health conditions, substance use, and lifestyle factors) and placental function.[75]

2.4 Maternal diseases and stress and the risk of CVD in the offspring

The prevalence of maternal chronic diseases during pregnancy has increased in recent decades in the Western countries,[13] a trend attributable to improved screening programs, increased incidence of several non-communicable diseases in the population, postponed motherhood, and medical progress enabling women with severe illnesses to carry pregnancies to term. There is a growing recognition that the well-being of women before or after conception, coupled with the subsequent foetal development, significantly influences the health of the offspring after birth. Increasing evidence from both experimental and observational studies suggests that various maternal conditions during pregnancy - including under- and over-nutrition, obesity, excessive weight gain, smoking, alcohol consumption, stress, chronic diseases/obstetric complications, inflammation, etc. - may increase CVD risk in the offspring irrespective of preterm birth or abnormal foetal growth.[37,80,81]

2.4.1 Maternal hypertensive disorders during pregnancy

Hypertensive disorders during pregnancy (HDP), recognized as an important determinant of maternal and neonatal mortality and morbidity, complicate 5.2-8.2% of all pregnancies.[82] The incidence of HDP has been rising in recent decades,[83,84] potentially due to improved screening practices and an increased prevalence of risk factors, including high maternal age, assisted reproductive technologies, obesity, and diabetes.[85]

In addition to chronic hypertension (hypertension existing before pregnancy or before 20 weeks of gestation), HDP consist of gestational hypertension (hypertension occurring after 20 weeks of gestation with recovery within 12 weeks after delivery) and preeclampsia (new-onset hypertension after 20 weeks of gestation characterized by poor placental perfusion and presenting as a multisystem syndrome).[85]

Preeclampsia is an etiologically heterogeneous disorder with two distinct entities defined by the timing of onset: early- and late-onset preeclampsia.[86] Early-onset preeclampsia (before 34 weeks of gestation) is considered to be mainly a placental disease featuring placental vascular lesions, oxidative stress, and reduced perfusion, while late-onset preeclampsia (at or after 34 weeks of gestation) is regarded to be mainly a maternal disease, often occurring in women with constitutional factors such as multiple births, high body-mass index (BMI), and diabetes.[87] Notably, early-onset preeclampsia is linked to higher risks of stillbirth, foetal growth restriction, preterm birth, and intensive care than late-onset preeclampsia.[88]

In addition to the immediate impact of HDP on maternal and foetal health, increasing evidence suggests that maternal HDP may program long-term cardiovascular health in the offspring. Most studies in this area suggest that individuals exposed to maternal preeclampsia or other HDP have a worse cardiovascular risk profile than their normotensive counterparts in terms of high blood pressure, high BMI, and abnormalities in lipid and glucose metabolism during childhood and early adulthood.[89-92] However, empirical evidence concerning the association between maternal HDP and offspring's CVD, especially severe heart or cerebrovascular diseases, is limited and inconsistent. A recent study in Denmark, including over 2 million live births from 1977 to 2018 with up to 42 years of follow-up, reported that individuals prenatally exposed to maternal HDP had higher risks of developing CVD and some of its subtypes, including myocardial infarction, stroke, AF, and HF.[93] There are also studies conducted in Finland,[94] US,[95] and Israel[96] reporting associations between maternal HDP and increased CVD (including specific subtypes like stroke) morbidity or mortality risk in children. However, other studies yielded different results. A previous Danish study with a maximum of 27 years follow-up,[97] did not find any association between maternal preeclampsia and offspring's CVD risk, potentially due to the rarity of CVD in young ages. The Northern Finland cohort study[98] found no link between maternal HDP and offspring's stroke; however, caution is warranted when ruling out a possible association given its limited statistical power.

The severity and time of onset of HDP, especially preeclampsia, may involve different underlying mechanisms. The result from the Helsinki study suggested that only individuals born to women with severe preeclampsia, not those with non-severe preeclampsia, had an increased risk of stroke.[94] Likewise, a Danish study observed the highest risk of overall CVD in the case of severe preeclampsia and early-onset preeclampsia.[93] Nevertheless, this study could not perform detailed analyses regarding the associations of preeclampsia subtypes with important types of CVDs, mainly due to the limited number of cases within subgroups.

Furthermore, associations between maternal HDP and subsequent CVD risk in the offspring become complex when considering foetal growth restriction and preterm birth. As mentioned above, severe HDP, particularly early-onset preeclampsia, is accompanied by preterm delivery and often by foetal growth restriction, both of which are linked to cardiovascular complications in the offspring. To date, few studies have thoroughly investigated whether the previously observed association between maternal HDP and CVD risk in children could be explained by foetal growth restriction and preterm

birth. Nevertheless, there is some preliminary evidence suggesting that offspring from hypertensive pregnancies may present cardiovascular dysfunction that cannot be attributed to prematurity or growth restriction only.[99,100]

The mechanisms underlying the link between maternal HDP and CVD risk in the offspring are not yet clear. The adverse intrauterine environment created by HDP may increase the risk of CVD through its impact on foetal programming. The hypertensive intrauterine environment may be characterized by placental insufficiency, endothelial dysfunction, hypoxia, increased level of anti-angiogenic factors, oxidative stress, and inflammation.[101] These *in-utero* insults have diverse effects on the foetus, including changes in the RAAS and immune system, cardiac-endothelial dysfunction and remodelling, which may influence foetal susceptibility to subsequent CVD.[102] In addition, the association between prenatal exposure to HDP and cardiovascular health might be partly explained by genetic, shared familial environmental and lifestyle factors.[102] Several genetic variants have been identified that are shared between preeclampsia and CVD, which may predispose mothers to preeclampsia and offspring to develop CVD.[103] Similarly, suboptimal environmental factors, such as low socioeconomic status and unhealthy lifestyle habits shared by mothers and their children, could contribute to the association of maternal HDP with the CVD risk in offspring.[102]

2.4.2   Maternal polycystic ovary syndrome

As a common endocrine disorder among women of reproductive age, polycystic ovary syndrome (PCOS) has a global prevalence ranging from 4% to 21%.[104,105] Though there are variations in diagnostic criteria, PCOS is characterized primarily by hyperandrogenism, ovarian dysfunction, menstrual irregularities, and reduced fertility due to oligomenorrhea.[104] In addition to reproductive abnormalities, women with PCOS have also a compromised cardiovascular risk profile, including insulin resistance, [106] type 2 diabetes,[107] obesity or overweight,[108] atherogenic dyslipidemia,[109] hypertension,[110] and atherosclerotic diseases.[111] The aetiology of PCOS remains unclear, with genetic and environmental factors playing roles in its development.[112]

During pregnancy, women with PCOS have an increased susceptibility to complications including endothelial dysfunction, gestational diabetes, gestational hypertension, and preeclampsia; these complications may influence foetal development and increase the risk of preterm delivery, SGA, LGA, and perinatal mortality.[113-115] Since several of these adverse pregnancy outcomes are associated with offspring's CVD risk after birth, a negative cardiovascular effect on the offspring of women with PCOS may also be hypothesised. Most studies in this area have focused primarily on metabolic, reproductive, or neuropsychiatric outcomes in the exposed offspring.[116] Only a limited number of studies have investigated the cardiovascular outcomes associated with being born to a mother with PCOS. A recent meta-analysis of nine observational studies showed signs of an unfavourable cardiometabolic risk profile, including increased levels of insulin resistance, low density lipoprotein cholesterol, and triglyceride, in children of women with PCOS.[117] A Dutch cohort study observed that children of women with PCOS had an increased pulse pressure and a higher left ventricular internal diameter at the age of 2.5 to 4 years and a higher carotid intima-media thickness (a marker of preclinical atherosclerosis) at the age of 6 to 8 years, indicating that the children of women with PCOS have subtle arterial dysfunction and may develop CVD later in life.[118] To the best of our knowledge, only two studies have investigated the direct link between maternal PCOS and offspring's CVD risk;

they reported an approximately 27%-31% increased risk of CVD in exposed offspring.[119,120] However, the small cohort sizes of both studies precluded thorough analyses of the effect of maternal PCOS on specific CVD types.

Several uncertainties persist in understanding the link between maternal PCOS and offspring's CVD, requesting large, well-designed studies with a sufficiently long follow-up. First, women with PCOS are more likely to have other comorbidities like hypertension,[110] obesity,[108] diabetes,[107] and psychological stress,[121] or to undergo assisted reproductive treatment,[122] all of which have been found to be linked to their children's CVD risk.[93,123-126] It is thus challenging to distinguish the effect of PCOS from that of other comorbidities and treatments. Second, it is unknown whether preterm birth, abnormal foetal growth, or subsequent cardiometabolic disorders mediate the potential association between maternal PCOS and the offspring's risk of CVD. Future studies confirming the mediating roles of these factors would shed light on the underlying mechanisms behind such an association. Third, it is highly plausible that maternal PCOS impacts children's cardiovascular health through intrauterine programming since high insulin and androgen levels in women with PCOS may contribute to an adverse intrauterine environment.[116] However, before establishing whether intrauterine programming by PCOS is the primary driver of an eventual maternal PCOS-offspring's CVD association, the contributions of shared familial genetic predisposition and lifestyle factors need to be examined.

2.4.3   Maternal stress during pregnancy

Prenatal exposure to severe maternal stress, frequently studied in terms of adverse life events, represents another adverse intrauterine exposure that may affect the offspring's short and long-term development.[127] Research investigating the risks of foetal outcomes associated with maternal stress has yielded mixed findings.[128] Some researchers observed associations between severe maternal stress and risks of stillbirth, congenital heart disease, foetal growth restriction, preterm birth, and neonatal mortality.[129-135] Conversely, other investigations within this filed did not observe such associations.[136,137] The majority of the studies investigating the association between maternal stress and the offspring's cardiovascular risk profile suggest that children of mothers experiencing adverse life events such as bereavement and natural disasters during pregnancy have increased risks of high blood pressure,[138] overweight and obesity,[139,140] and type 2 diabetes.[141] In contrast, others found weak or no association between prenatal exposure to severe maternal stress and cardiometabolic outcomes or the vascular function.[142,143]

Knowledge about the relationship between prenatal exposure to severe maternal stress and the subsequent risk of CVD is limited. Two studies from the Dutch Famine Birth Cohort (1944-45) reported an increase in IHD risk in adults exposed to the famine prenatally,[144,145] while another Dutch study did not find such a association.[146] On the other hand, the Helsinki Birth Cohort Study (1934-44) found that individuals who prenatally experienced bombings during World War II had a lower risks of IHD and stroke after the age of 50 than unexposed individuals.[147] However, a methodological concern with these historical cohort studies is that ecological measures of famine and wartime may not be precise indicators of stress, as they operate at an ecological level. These exposures can encompass not only stress but also factors like malnutrition, toxic materials and restricted healthcare access, which can additionally impact foetal development. Moreover, survival bias may exist in these earlier studies,

as those who survived extreme circumstances like famine and war could be more resilient and may have better health.

The loss of a close family member is rated as one of the most severe stressors by several well-known classification systems of stress.[148,149] It could serve as a more suitable proxy for stress than using war or famine as a stressor, as it is likely to be perceived as stressful by everyone regardless of coping skills and social support.[150] Adjustment to certain types of losses may be particularly difficult. The loss of a spouse or child are considered to be more severe than the loss of other relatives, while losses due to unnatural, sudden, and unexpected death, may induce more stress than natural death.[149] Therefore, using bereavement as an exposure allows researchers to analyse the dose-response relationship between prenatal stress and CVD outcomes. A Danish population-based study observed a modest relationship between maternal bereavement and the offspring's overall CVD risk, both in childhood and early adulthood.[151] However, the association disappeared after controlling for shared familial confounders. A recent study by our group found no link between maternal bereavement and offspring's risks of stroke and IHD up to the age of 48 years.[152]

Several plausible pathways have been noted through which maternal stress during pregnancy may affect the offspring's cardiovascular health. One well-described mechanism involves changes in the maternal/foetal HPA axis in response to maternal stress and the consequent adaptation of glucocorticoid hormone production during pregnancy.[153] The activated HPA axis, in turn, may influence the function of the foetal cardiovascular system. Maternal stress could also disrupt the function of foetal RAAS and the autonomic nervous system, potentially contributing to an increase in blood pressure and heart rate variability in later life.[154] Other potential mechanisms include inflammatory responses, oxidative stress, adverse epigenetic changes, and the indirect effects of maternal behaviour changes.[127]

2.5 Knowledge gaps

This literature review reveals that substantial knowledge has accumulated concerning the association between prenatal risk factors and the risk of CVD later in life. However, several knowledge gaps remain:

1. **Adverse birth outcomes and AF:**
   While there is a growing focus on the association between adverse birth outcomes and the risk of developing CVD later in life, limited and inconsistent knowledge exists regarding AF. Additionally, the few studies in this area focused on individual effects of abnormal foetal growth or preterm birth, neglecting investigations into their combinations on AF risk.

2. **Maternal diseases and severe stress and specific CVD types:**
   Offspring born to mothers with conditions such as preeclampsia, overall and its subtypes, and PCOS, or severe maternal stress have been reported to have an adverse cardiovascular risk profiles. However, evidence regarding their associations with the risk of developing CVD, particularly specific severe types of CVD, remains limited.

3. **Mechanisms underlying these associations:**
   The relative roles of *in-utero* insult programming and genetic traits in the association between prenatal risk factors and later CVD risk are not fully elucidated. Unanswered questions

revolve around whether the association results from suboptimal *in-utero* environments, genetic predisposition, or their combination. Additionally, the contribution of abnormal birth outcomes to the association between maternal diseases or severe stress and the risk of CVD in offspring remains unclear.

The four studies in this thesis attempted to contribute to filling these knowledge gaps.

3 RESEARCH AIMS

The objective of this thesis was to enhance understanding regarding the role of prenatal risk factors in the development of CVD later in life. The specific research questions are shown in Figure 3.1.
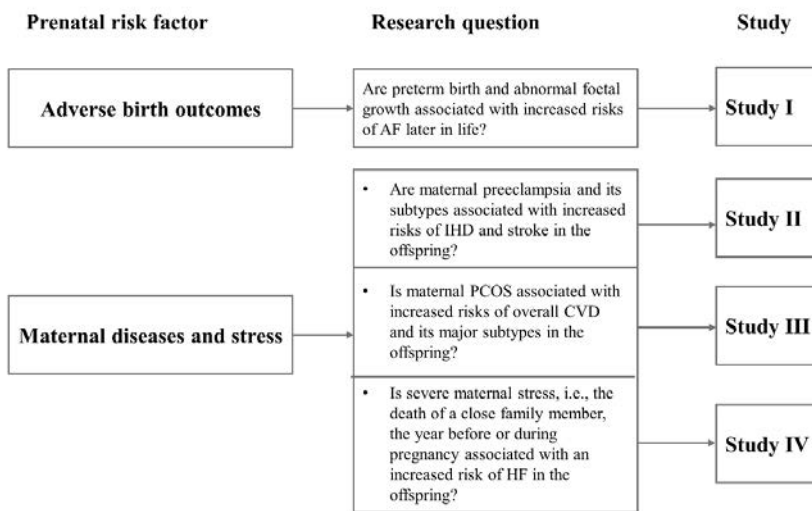
Figure 3.1 The summary of the research questions investigated in Studies I-IV

Abbreviations: AF, atrial fibrillation; IHD, ischemic heart disease; PCOS, polycystic ovary syndrome; CVD, cardiovascular disease; HF, heart failure.

4 MATERIALS AND METHODS

4.1 Overview

Table 4.1. The summary of study design, population, and main measures in Studies I-IV

	Study I (N=8,012,433)	Study II (N=8,475,819)	Study III (N=6,839,703)	Study IV (N=6,758,560)
Study design	Nationwide population-based cohort study			
Population	Singletons born alive in Denmark between 1978 and 2016, in Finland between 1987 and 2014, and in Sweden between 1973 and 2014	Singletons born alive in Denmark between 1973 and 2016, in Finland between 1987 and 2014, and in Sweden between 1973 and 2014	Singletons born alive in Denmark between 1973 and 2016 and in Sweden between 1973 and 2014	
Follow-up period	From birth to the end of 2016 in Denmark, of 2014 in Finland, and of 2020 in Sweden	From birth to the end of 2016 in Denmark and of 2014 in Finland and Sweden	From birth to the end of 2016 in Denmark and of 2020 in Sweden	
Exposures	Preterm birth, small and large for gestational age	Maternal preeclampsia	Maternal polycystic ovary syndrome	Severe maternal stress (using bereavement as a proxy)
Outcomes	Atrial fibrillation	Ischemic heart disease and stroke	Overall cardiovascular disease and its major subtypes	Heart failure
Statistical analyses	Cox regression model			
Family design	Sibling comparison		Cousin comparison	Not applicable

4.2 Data source

The thesis comprises four register-based studies conducted by linking data from Danish, Finnish, and Swedish registries; linkage was possible through the participants' unique identification numbers. Given the data available to us for each specific research question, we employed information from all three countries for Studies I and II, whereas for Studies III and IV, only data from Denmark and Sweden were utilized. The detailed description of registries included in our four studies and the variables retrieved from registries are presented in Table 4.2.

Table 4.2. Description of the registers used in Studies I-IV*

Register	Information	Period covered
Denmark		
Danish Civil Registration System	Sex, date of birth, place of birth, vital status, marital status, migration, and linkage to parents and siblings	1968-2016
Danish Integrated Database for Labour Market Research	Mother's highest completed education	1980-2016
Danish Medical Birth Register	Sex, date of birth, gestational age, birth weight, singleton status, mother's body mass index and smoking in early pregnancy, age at delivery, and parity	1973-2016
Danish National Patient Register	Inpatient and outpatient care (diagnosis, date)	Inpatient care: 1977-2016; outpatient care and emergency department contacts: 1995-2016
Danish Register of Causes of Death	Date of death and cause of death	1970-2016
Finland		
Finnish Central Population Register	Sex, date of birth, vital status, marital status, and migration	1968-2014

Education Register at Statistics Finland	Mother's highest completed education	1970-2014
Finnish Medical Birth Register	Sex, date of birth, sex, gestational age, birth weight, singleton status, mother's smoking in early pregnancy, age at delivery, and parity	1987-2014
Finnish Hospital Discharge Register	Information on inpatient and outpatient care (diagnosis, date)	Inpatient care: 1969-2014; outpatient care contacts: 1998-2014
Finnish Causes of Death Register	Date of death and cause of death	1969-2014

Sweden

Swedish Total Population Register	Sex, date of birth, place of birth, vital status, marital status, and migration	1968-2014
Swedish Multi-Generation Register	Relationships for all residents born since 1932	1961-2014
Swedish Register of Education	Mother's highest completed educational attainment	1985-2014
Swedish Medical Birth Register	Sex, date of birth, gestational age, birth weight, singleton status, mother's smoking and body mass index during pregnancy, mother's age at delivery, parity, and complications during pregnancy	1973-2014
Swedish Patient Register	Information on inpatient and outpatient care (diagnosis, date)	Inpatient care: 1964-2020 (its coverage became nationwide since 1987); outpatient care: 2001-2020
Swedish Cause of Death Register	Date and cause of death	1952-2020

[*] This table is adapted from eTable 1 in Yang F, Janszky I, Gissler M, et al. JAMA Network Open. 2022;5(11):e2242064,[155] with the permission from the publisher.

4.3 Study design and populations

4.3.1 General study design and populations for Studies I-IV

We conducted four population-based prospective cohort studies (Figure 4.1) based on the nationwide Danish, Finnish, and/or Swedish Medical Birth Registries. The study participants were all live singleton births from Denmark (Study I: 1978-2016, Studies II-IV: 1973-2016), and Sweden (Studies I-IV: 1973-2014), and singletons from a randomly selected group consisting of 90% of births in Finland (1987-2014, Studies I and II). We chose to include only singletons as clinical experience suggests that information on twins or multiples registered at birth cannot always securely be linked with their health information later in life, due to mixing of information between twins. We included only 90% of Finnish births because Statistics Finland does not allow 100% of the complete data to be extracted from the target population.

We followed each individual from birth until the first diagnosis of the CVD outcome of interest, death, emigration, or end of follow-up, whichever occurred first.
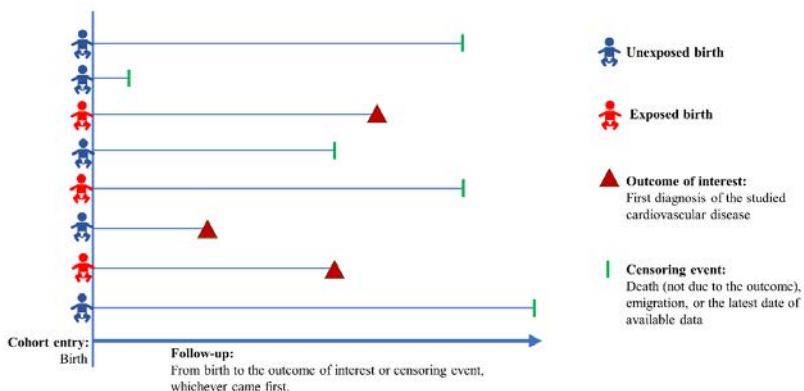
Figure 4.1 General study design for Studies I-IV

4.3.2 Specific population selection criteria for Studies I, III and IV

In Study I, we excluded study participants who had no information on sex, gestational age, or birth weight, as well as those with an implausible gestational age (<22 weeks or >45 weeks) or birth weight for gestational age (deviating more than five standard deviations from the mean for sex- and gestational age- specific value).

In Studies III and IV, we did not include births from Finland due to the unavailability of adequate information on PCOS and on the mother's family members, which was crucial for the conducted analyses.

4.4 Measurements

4.4.1 Exposures

For Study I, we defined our three exposures of interest, i.e., preterm birth, SGA, and LGA, based on data on sex, birth weight and gestational age, from the nationwide Medical Birth Registries. Gestational age was estimated through routine ultrasound examinations conducted during the early second trimester. It is worth noting that uniform ultrasound examinations were introduced in Denmark, Finland, and Sweden in the mid-1990s. In cases of missing ultrasound data, we used gestational age estimated based on the date of the last menstrual period, if available. Birth weight for gestational age was determined using the sex-specific reference curve for foetal weight developed by Maršál,[156] Subsequently, we transformed birthweight for gestational age into z-scores and calculated percentiles. We defined preterm birth as birth before 37 full gestational weeks and categorized it further into moderately preterm birth (32-36 gestational weeks) and extremely and very preterm birth (22-31 gestational weeks). The reference group was term birth (gestational age ≥37 weeks). We defined SGA and LGA as below the 10th percentile and above the 90th percentile, respectively. Further, we categorized them as severe SGA (<3rd percentile), moderate SGA (3rd to10th percentile), moderate LGA (90th to 97th percentile), and severe LGA (>97th percentile). The reference group was appropriate for gestational age (AGA) birth, representing birth weight for gestational age between the 10th and the 90th percentile.

In Study II, exposure was maternal preeclampsia during the index pregnancy. We obtained data on maternal preeclampsia from the Danish National Patient Register, the Finnish Hospital Discharge Register, and the Swedish Medical Birth Register, using the International Classification of Diseases (ICD) codes shown in Table 4.3. To assess the severity of maternal preeclampsia, we employed three distinct strategies: using ICD codes, the time of onset of preeclampsia, and the occurrence of foetal growth restriction. The details are presented in Table 4.4.

In Study III, the exposed group was defined as offspring whose mother ever received a diagnosis of PCOS, identified through the Danish National Patient Register, the Swedish Patient Register, and the Swedish Medical Birth Register (refer to Table 4.3 for the corresponding ICD codes). We considered PCOS to be present during pregnancy, irrespective of the time of diagnosis, given its nature as a lifelong disorder characterized by metabolic disturbances and consistently elevated testosterone levels that persist throughout the life of affected women.[157,158]

In Study IV, we defined prenatal stress as loss of a close family member- namely, a partner, child, parent, or siblings- one year before or during pregnancy. To identify mothers' family members, we used the Danish Civil Registration System and the Swedish Multi-generation Register. Additionally, we accessed information on the deceased's date and cause of death from the Danish Register of Causes of Death and the Swedish Cause of Death Register. In instances where more than one loss occurred during the exposure window, we included only the first loss in the analyses. We further categorized the exposed group according to:

- The time of the mother's loss (up to one year before pregnancy or during each of the three trimesters of pregnancy).
- The relationship between the mother and the deceased (older child or partner and parent or sibling).

- The cause of death for the deceased (death due to natural and unnatural causes, which were identified by the ICD codes in Table 4.3).

We regarded losses involving children or partners, or unnatural deaths as more severe sources of stress than other types of losses.[149]

4.4.2   Outcome

In Study I the outcome was AF (including atrial flutter). In Study II the outcomes were IHD and stroke (including separately also ischemic stroke and hemorrhagic stroke). In Study III, the outcomes encompassed overall CVD and main CVD subtypes such as AF, IHD, stroke, HF, and hypertensive diseases. The outcome of Study IV was HF.

To ascertain overall CVD and its specific types including stroke, IHD, and HF, we relied on the primary diagnosis in the national patient registers or the underlying cause of death in the cause of death register. The validity of these CVD outcomes in the patient registers and the cause of death registers has been demonstrated to be high.[159-164] Besides, hypertensive diseases, AF, and peripheral artery disease were identified based on primary or secondary diagnoses in national patient registers. Detailed ICD codes for CVD outcomes in Studies I-IV are provided in Table 4.3.

4.4.3   Covariates

In Studies I-IV, we selected covariates from the linked registers, as described in Table 4.2.

1) Maternal characteristics:

The information on the maternal country of origin and marital status before the index birth were obtained from the Danish Civil Registration System, the Finnish Central Population Register, and the Swedish Total Population Register. We obtained data on the highest education from the Danish Integrated Database for Labour Market Research, the Education Register at Statistics Finland, and the Swedish Register of Education. From the Danish, Finnish, and Swedish Medical Birth Registers, we extracted maternal age when giving birth, parity, body-mass index (BMI, available since 2003 in Denmark and since 1982 in Sweden), and smoking (available since 1991 in Denmark, since 1987 in Finland, and since 1982 in Sweden) in early pregnancy, and assisted reproductive treatment (included only in Study III, available since 1994 in Denmark and since 1995 in Sweden) during the index pregnancy. Information on maternal hypertensive disease, diabetes, and psychiatric disorders before or during the index pregnancy was obtained from the Danish National Patient Registry, the Finnish Hospital Discharge Register, and the Swedish Medical Birth Register (using the ICD codes in Table 4.3). We also collected data on the family history of CVD, i.e., CVD diagnoses of the mother's parents and siblings, from national patient registers.

2)   Offspring characteristics:

Data on the offspring's country and birth year, sex, gestational age, and birth weight were extracted from the Danish, Finnish, and Swedish Medical Birth Registers. The information on congenital anomalies, including congenital heart diseases, diabetes, and some major CVD types such as

hypertensive diseases, IHD, stroke, and HF, were extracted from the national patient registries (refer to Table 4.3 for corresponding ICD codes).

Table 4.3. International Classification of Diseases codes searched for to define variables in Studies I-IV

	ICD-8	ICD-9	ICD-10
Preeclampsia			
Denmark [a]	637.03, 637.04, 637.09, 637.19		O14, O15
Finland [b]		6424, 6425, 6426	O14, O15
Sweden [c]	637.03, 637.04, 637.09, 637.99, 637.10	642E, 642F, 642G	O14, O15
Mild to moderate preeclampsia			
Denmark [a]	637.03, 637.09		O14.0, O14.9
Finland [b]		6424	O14.0, O14.9
Sweden [c]	637.03, 637.09, 637.99	642E	O14.0, O14.9
Severe preeclampsia			
Denmark [a]	637.04, 637.19		O14.1, O14.2, O15
Finland [b]		6425, 6426	O14.1, O15
Sweden [c]	637.04, 637.10	642F, 642G	O14.1, O14.2, O15
Pregestational diabetes and gestational diabetes mellitus			
Denmark [a]	250		E10-E14, O24
Finland [b]		250, 6480A, 6488A	E10-E14, O24
Sweden [c]	250	250, 648A, 648W	E10-E14, O24

Polycystic ovary syndrome [d]

Denmark [a]	25690		E282
Sweden [c]	25690	256E	E282

Overall cardiovascular disease [d]

Denmark [a]	390-458		I00-I99
Sweden [c]	390-458	390-459	I00-I99

Ischemic heart disease

Denmark [a]	410-414		I20-I25
Finland [b]		4100, 4109, 4110, 4120, 4121, 4131, 4140, 4148, 4149	I20-I25
Sweden [c]	410-414	410-414	I20-I25

Stroke

Denmark [a]	430, 431, 433, 434, 436		I60, I61, I63, I64
Finland [b]		430, 431, 433, 434, 436	I60, I61, I63, I64
Sweden [c]	430, 431, 433, 434, 436	430, 431, 433, 434, 436	I60, I61, I63, I64

Atrial fibrillation

Denmark [a]	427.93, 427.94		I48
Finland [b]	427.92	427D	I48
Sweden [c]		4273	I48

Heart failure

Denmark [a]	42709, 42710, 42711, 42719		I110, I130, I132, I50

Finland [b]		428	I11.0, I13.0, I13.2, I50
Sweden [c]	427.00, 427.10	428	I11.0, I13.0, I13.2, I50

Congenital anomalies

Denmark [a]	740-759		Q00-Q99
Finland [b]		740-759	Q00-Q99
Sweden [c]	740-759	740-759	Q00-Q99

Congenital heart disease

Denmark [a]	746, 747		Q20-Q27
Finland [b]		745, 746, 747	Q20-Q28
Sweden [c]	746, 747	745, 746, 747	Q20-Q28

Diabetes

Denmark [a]	250		E10-E14
Finland [b]		250	E10-E14
Sweden [c]	250	250	E10-E14

Psychiatric disorders [d]

Denmark [a]	290-315		F00-F99
Sweden [c]	290-315	290-319	F00-F99

Unnatural death [d]

Denmark [a]	795, E800-E999		R95-R97, V00-Y99
Sweden [c]	7959, 79621, E800-E999	798, E800-E999	R95, R96, R98, V01-Y98

Abbreviations: ICD, International Classification of Diseases.

[a] We used the Danish version of ICD-8 up to 1995 and ICD-10 afterwards.
[b] We used the Finnish version of ICD-9 up to 1995 and ICD-10 afterwards.

d Information on these diseases was specifically extracted from Danish and Swedish registers for the purposes of Studies III and IV.

Table 4.4. Three classification strategies for assessing the severity of maternal preeclampsia

Classification strategies	Severity of preeclampsia	
	Mild forms	**Severe forms**
According to ICD codes	Mild to moderate preeclampsia	Severe preeclampsia
According to the time of onset [a]	Late-onset preeclampsia (based on a preeclampsia diagnosis given at or after gestational week 34)	Early-onset preeclampsia (based on a preeclampsia diagnosis given before gestational week 34)
According to the co-occurrence of foetal growth restriction	Preeclampsia in a pregnancy without a small for gestational age birth	Preeclampsia in a pregnancy with a small for gestational age birth

Abbreviations: ICD, International Classification of Diseases

a Due to the absence of information on the diagnosis date of preeclampsia in the Swedish Medical Register, we identified mothers with a preeclampsia diagnosis who delivered their child prior to 34 weeks of gestation as having early-onset preeclampsia.

4.5   Statistical analyses

4.5.1   Main analysis for Studies I-IV

We ran Cox regression models to analyze the associations between our prenatal risk factors and the specific CVD outcomes; in all models we used attained age in years as the underlying time scale. There was evidence for violation of the assumption of proportional hazards, as assessed using log-log survival plots and Schoenfeld's residuals,[165] in all studies, except in Study III. To address non-proportionality of the exposure in Studies I, II, and IV, we performed analysis after splitting the follow-up period and calculating risk estimates in different time intervals. Further details are provided in Section 4.5.2.

In Study I, we first modeled birth weight for gestational age and gestational age as continuous variables and fitted them in the Cox model with restricted cubic spline functions. This approach allowed exploring potential non-linearity of the dose-response relationships between these variables and the AF risk. We further applied the Cox model to estimate the associations for AF with the three categorical exposures: preterm birth, SGA (including severe and moderate SGA), and LGA (including severe and moderate LGA). Moreover, we analysed the joint effects of these exposures by estimating HRs for five subgroups: term SGA birth, term LGA birth, preterm SGA birth, preterm AGA birth, preterm LGA birth, with the reference group as term AGA birth.

In Study II, we first employed Cox regression models to estimate the risks of IHD and stroke associated with prenatal exposure to maternal preeclampsia. Next, we analysed whether the IHD and stroke risks varied based on the severity of preeclampsia, defined as: 1) early-onset vs. late-onset preeclampsia; 2) severe vs. mild/moderate preeclampsia (according to the ICD codes); and 3) preeclampsia with vs. without SGA.

In Study III, we applied Cox regression models to estimate the associations between maternal PCOS and the risks of overall CVD and some of its major subtypes, including hypertensive diseases, IHD, stroke, AF, and HF. In addition, given that mothers with PCOS often have comorbidities such as obesity,[108] diabetes,[166] hypertension,[167] and psychiatric disorders,[121] we further examined the joint effects of maternal PCOS and these comorbidities on the risk of CVD in offspring.

In Study IV, we analysed the association between severe maternal stress and HF risk in offspring using Cox regression models. We further analysed whether the HRs for HF varied by the time of loss (up to one year before pregnancy, and during each of the three trimesters of pregnancy), the mother's relationship to the deceased (older child or partner versus parent or sibling), and the cause of death of the deceased family member (unnatural death versus natural death).

In the multivariable Cox model conduced in Studies I-IV, we controlled for country, sex (except for SGA and LGA analyses in Study I), birth weight (only for preterm birth analyses in Study I), and calendar year of birth, and maternal characteristics including country of origin, parity, age, education and marital status at delivery, BMI and smoking in early pregnancy, maternal hypertensive disease (except for Study II), diabetes, psychiatric disorders (included only in Studies III and IV), and family history of CVD before delivery. Due to lack of available data for some study participants, we conducted sensitivity analyses that accounted for family history of CVD, maternal country of origin (in the case of Studies I and II), and smoking and BMI in early pregnancy only among those with available data.

4.5.2   Sub-analyses for Studies I-IV

1) Stratified analyses by different time intervals

To account for the non-proportional HRs in Studies I, II, and IV and also explore associations across distinct life periods for all studies, we performed stratified analyses by splitting the follow-up time at the age of 18 years, into childhood and adolescence (attained age<18 years) and young adulthood (attained age≥18 years).

2) Sibling and cousin comparison analyses

To consider the influence of unmeasured familial confounders, including genetic and environmental risk factors, we implemented a family design approach involving sibling-comparison analyses for Studies I and II and cousin-comparison analyses for Study IV. In brief, we performed stratified Cox regression models with a separate stratum for each sibling pair (for sibling comparison), identified by the mother's personal identification number, or cousin pair (for cousin comparison), identified by the mother and her mother's and biological sister's personal identification numbers. Only sibling and cousin pairs discordant for exposure and outcome contributed to the estimates in these analyses.

3) Mediation analyses

Maternal PCOS and severe stress during pregnancy have been associated with adverse outcomes in offspring, including preterm birth, abnormal foetal growth (SGA or LGA births), and congenital heart disease in offspring.[115,119,131,134,168] These outcomes are potentially risk factors for CVD.[14,15,169] Therefore, in Studies III and IV, we conducted mediation analyses to explore the roles of preterm birth, SGA or LGA birth, and congenital heart disease in the association between maternal PCOS or severe stress and offspring's CVD outcomes.

In our mediation analyses, grounded in the counterfactual framework,[170] we estimated the total effect of maternal conditions (i.e., PCOS and severe stress) on CVD risks, the direct effect (representing the effect of maternal conditions on CVD risks independent of these mediators), and the mediated effect (representing the effect of maternal conditions on CVD risks through their effect on the investigated mediators). We further quantified the proportion mediated by each mediator based on these effect estimates. A 0% indicated no mediated effect, while 100% indicated no direct effect. This analytical approach allowed us to attempt to discern the intricate interplay between maternal conditions, perinatal outcomes, and the subsequent development of CVD risks in offspring.

4.6   Ethical considerations

Our research is based on register data from Danish, Finnish, and Swedish registers, and has been conducted in line with medical ethical principles, i.e., autonomy, beneficence, non-maleficence, and justice.

The primary ethical considerations associated with the project pertain to the confidentiality of information concerning study participants. After approval from the Danish Data Protection Agency (No. 2013-41-2569) and the Research Ethics Committee at Karolinska Institute in Stockholm (No. 2016/288-31/1, 2021-03315), the project coordinators from each country sent requests for data collection to the main register holders (government agencies) in the three countries. The main register holders made the linkage between registries, replacing personal identification numbers with encrypted counterparts before transferring the data to our coordinators. Consequently, we only have access to pseudonymized data, restricting the use of these data sources exclusively to the index project. The collected data are stored on the secure platform of Statistics Denmark, to which only research group members have access. All researchers in our project are informed thoroughly about the rules concerning data protection before getting access to the secure platform. To minimize the risk of identifying specific study participants, we aggregated sub-groups with few cases when presenting results with extreme values, safeguarding individual privacy. As a result, data security risks in our research are mitigated through stringent rules.

Another ethical concern in our project relates to informed consent. According to the strict regulation rules on data protection in register-based research, researchers are not allowed to contact study participants and have no contact information on subjects, which means that it is not possible to obtain informed consents from them. Accordingly, there is no request for informed consent in case of register-based research in Nordic countries. We believe that the potential knowledge gains from our project would outweigh any possible harms to the study participants as the latter ones are likely to be negligible.

5 RESULTS

5.1 Associations between adverse birth outcomes and the AF risk (Study I)

In Study I, the prevalence rates of preterm birth, SGA, LGA, and AF were 4.7%, 10.0%, 10.0%, and 0.14%, respectively. The incidence rates of AF were 0.82, 0.65, 0.69, 0.88, and 0.63 per 10,000 person-years for individuals with preterm birth, term birth, SGA birth, LGA birth, and AGA birth, respectively.

Our analysis revealed a decreasing risk of AF with increasing gestational age, while the risk increased with higher birth weight for gestational age (Figure 5.1).
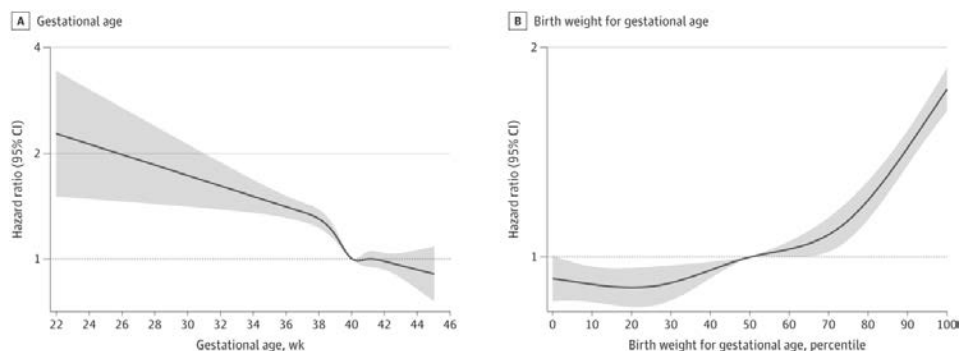

Figure 5.1. Adjusted hazard ratios and 95% confidence intervals corresponding to the association between gestational age (A) and birth weight for gestational age (B) and the risk of atrial fibrillation using the Cox model with restricted cubic splines

The models were controlled for country, year of birth, sex and birth weight (only included in the Cox model for gestational age), and maternal age, marital status, education, parity, diabetes and hypertensive disorders prior to the index childbirth. This figure is adapted from Yang F, Janszky I, Gissler M, et al. JAMA Pediatrics. 2023;177(6):599–607,[171] with the permission of the publisher.

An association between preterm birth and the risk of AF was observed in both the population and the sibling analyses. Effects sizes were higher, in childhood/adolescence (attained age < 18 years) than in adulthood (attained age ≥ 18 years) (Figure 5.2). When investigating categories of preterm birth, only moderately preterm birth presented an association with an increased AF risk.

In the population analysis, individuals born SGA had a lower risk of AF than those born AGA. In contrast, those born LGA had a higher AF risk than the those born AGA, with stronger associations for severe LGA than for moderate LGA (Figure 5.2). In the sibling analysis, the association persisted only for LGA. It is noteworthy that persons born SGA had an elevated risk of AF during childhood but not in adulthood, whereas the association between LGA and AF remained consistent across the two periods.

When investigating the joint effect of preterm birth and SGA or LGA, we found that, compared with persons born at term with AGA, those born preterm with LGA had the highest AF risk (adjusted HR:1.71, 95% CI: 1.40-2.09), followed by those born at term with LGA (adjusted HR:1.55, 95% CI: 1.47-1.64), those born preterm with AGA (adjusted HR:1.31, 95% CI: 1.10-1.56), and those born preterm with SGA (adjusted HR:1.25, 95% CI: 1.14-1.38).

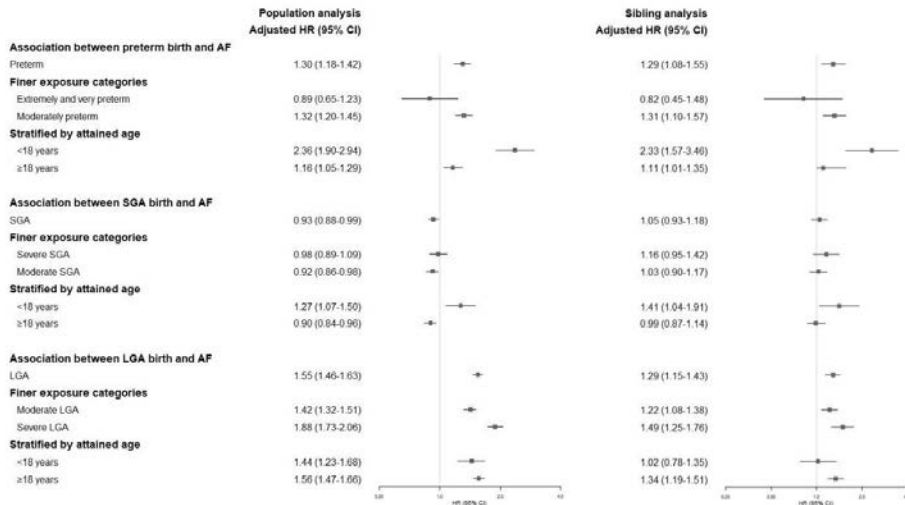|	Population analysis Adjusted HR (95% CI)		Sibling analysis Adjusted HR (95% CI)	
Association between preterm birth and AF				
Preterm	1.30 (1.18-1.42)		1.29 (1.08-1.55)	
Finer exposure categories				
Extremely and very preterm	0.89 (0.65-1.23)		0.82 (0.45-1.48)	
Moderately preterm	1.32 (1.20-1.45)		1.31 (1.10-1.57)	
Stratified by attained age				
<18 years	2.36 (1.90-2.94)		2.33 (1.57-3.46)	
≥18 years	1.16 (1.05-1.29)		1.11 (1.01-1.35)	
Association between SGA birth and AF				
SGA	0.93 (0.88-0.99)		1.05 (0.93-1.18)	
Finer exposure categories				
Severe SGA	0.98 (0.89-1.09)		1.16 (0.95-1.42)	
Moderate SGA	0.92 (0.86-0.98)		1.03 (0.90-1.17)	
Stratified by attained age				
<18 years	1.27 (1.07-1.50)		1.41 (1.04-1.91)	
≥18 years	0.90 (0.84-0.96)		0.99 (0.87-1.14)	
Association between LGA birth and AF				
LGA	1.55 (1.46-1.63)		1.29 (1.15-1.43)	
Finer exposure categories				
Moderate LGA	1.42 (1.32-1.51)		1.22 (1.08-1.38)	
Severe LGA	1.88 (1.73-2.06)		1.49 (1.25-1.76)	
Stratified by attained age				
<18 years	1.44 (1.23-1.68)		1.02 (0.78-1.35)	
≥18 years	1.56 (1.47-1.66)		1.34 (1.19-1.51)	

Figure 5.2. Adjusted hazard ratios and 95% confidence intervals for the association between preterm birth, small and large for gestational age with the risk of atrial fibrillation

Abbreviations: HR, hazard ratio; CI, confidence interval; AF, atrial fibrillation; SGA, small for gestational age; LGA, large for gestational age. Adjustment was made for country, year of birth, sex and birth weight (only in analyses of preterm birth), and maternal age, education, marital status, parity, diabetes and hypertensive disorders prior to the index childbirth.

5.2 Associations between maternal diseases and stress and the risk of CVD in offspring (Studies II-IV)

5.2.1 Associations between maternal preeclampsia and risk of IHD and stroke in the offspring (Study II)

In Study II, 2.2% of the study participants were born to mothers with preeclampsia, 0.1% had a diagnosis of IHD, and 0.1% had a diagnosis of stroke. The incidence rates of IHD among exposed offspring (i.e., born after a pregnancy with preeclampsia) and those unexposed (i.e., born after a normotensive pregnancy) were 0.42 and 0.46 per 10,000 person-years, respectively. For stroke, the incidence rates among exposed and unexposed offspring were 0.74 and 0.66 per 10,000 person-years, respectively.

Study participants whose mother had preeclampsia while pregnant with them had a higher risk of IHD (adjusted HR, 1.33; 95% CI: 1.12-1.58) and stroke (adjusted HR, 1.34; 95% CI: 1.17-1.52) compared to individuals born to normotensive mothers, as illustrated in Figure 5.3. Furthermore, the risk estimates for both outcomes were higher among offspring of mothers with severe forms of preeclampsia (including early-onset preeclampsia, severe preeclampsia, or preeclampsia with SGA) than those with mild forms (including late-onset preeclampsia, mild/moderate preeclampsia, preeclampsia without SGA). When conducting sibling comparison analyses, most adjusted HRs for IHD and stroke were attenuated. However, the estimates for stroke were consistently high in cases of severe forms of preeclampsia (Figure 5.3).

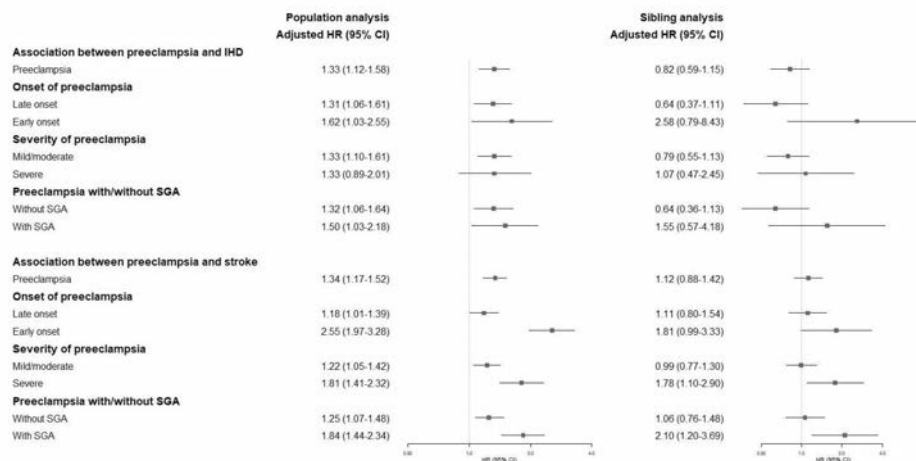|	Population analysis Adjusted HR (95% CI)		Sibling analysis Adjusted HR (95% CI)	
Association between preeclampsia and IHD				
Preeclampsia	1.33 (1.12-1.58)		0.82 (0.59-1.15)	
Onset of preeclampsia				
Late onset	1.31 (1.06-1.61)		0.64 (0.37-1.11)	
Early onset	1.62 (1.03-2.55)		2.58 (0.79-8.43)	
Severity of preeclampsia				
Mild/moderate	1.33 (1.10-1.61)		0.79 (0.55-1.13)	
Severe	1.33 (0.89-2.01)		1.07 (0.47-2.45)	
Preeclampsia with/without SGA				
Without SGA	1.32 (1.06-1.64)		0.64 (0.36-1.13)	
With SGA	1.50 (1.03-2.18)		1.55 (0.57-4.18)	
Association between preeclampsia and stroke				
Preeclampsia	1.34 (1.17-1.52)		1.12 (0.88-1.42)	
Onset of preeclampsia				
Late onset	1.18 (1.01-1.39)		1.11 (0.80-1.54)	
Early onset	2.55 (1.97-3.28)		1.81 (0.99-3.33)	
Severity of preeclampsia				
Mild/moderate	1.22 (1.05-1.42)		0.99 (0.77-1.30)	
Severe	1.81 (1.41-2.32)		1.78 (1.10-2.90)	
Preeclampsia with/without SGA				
Without SGA	1.25 (1.07-1.48)		1.06 (0.76-1.48)	
With SGA	1.84 (1.44-2.34)		2.10 (1.20-3.69)	

Figure 5.3. Adjusted hazard ratios and 95% confidence intervals for the association between maternal preeclampsia, overall and by subtype, and the risk of ischemic heart disease and stroke

Abbreviations: HR, hazard ratio; CI, confidence interval; IHD, ischemic heart disease; SGA, small for gestational age. We controlled for sex, country, birth year, and maternal age, education, marital status, parity, and diabetes prior to the index childbirth.

When splitting follow-up at the age of 18 years, we observed a stronger association between preeclampsia and stroke in childhood/adolescence than in adulthood. In contrast, the association with IHD was observed only in adulthood (Figure 5.4).
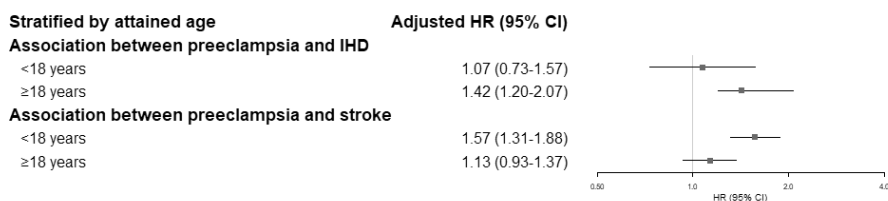
Stratified by attained age	Adjusted HR (95% CI)	
Association between preeclampsia and IHD		
<18 years	1.07 (0.73-1.57)	
≥18 years	1.42 (1.20-2.07)	
Association between preeclampsia and stroke		
<18 years	1.57 (1.31-1.88)	
≥18 years	1.13 (0.93-1.37)	

HR (95% CI)

Figure 5.4. Hazard ratios and 95% confidence intervals for the association between maternal preeclampsia and ischemic heart disease and stroke in offspring, by attained age

Abbreviations: HR, hazard ratio; CI, confidence interval; IHD, ischemic heart disease. We controlled for sex, country, birth year, and maternal age, education, marital status, parity, and diabetes prior to the index childbirth.

5.2.2 Association between PCOS in the mother and risks of CVD and its major types in the offspring (Study III)

In Study III, 0.76% of study participants were born to mothers with PCOS, and 5.6% of study participants had a diagnosis of CVD. The incidence rates of CVD among individuals born to mothers with PCOS and those born to mothers without PCOS were 22.41 and 23.93 per 10,000 person-years, respectively.

In the population analysis, offspring born to mothers with PCOS had higher risks of CVD (adjusted HR: 1.21; 95% CI: 1.15-1.27) and some specific CVD types, including hypertensive disease, IHD, and stroke, compared to those born to mothers without PCOS (Figure 5.5). We did not observe any association of maternal PCOS with risks of AF and HF. In the cousin comparison analysis, the associations between maternal PCOS and risks of CVD, hypertensive disease, and IHD were very similar to the associations observed in the population analysis (Figure 5.5).

When splitting follow-up at the age of 18 years, we found that the strength of the association between maternal PCOS and overall CVD in childhood/adolescence (adjusted HR: 1.19, 95% CI: 1.12-1.26) was similar to that in adulthood (adjusted HR: 1.22, 95% CI: 1.11-1.34).
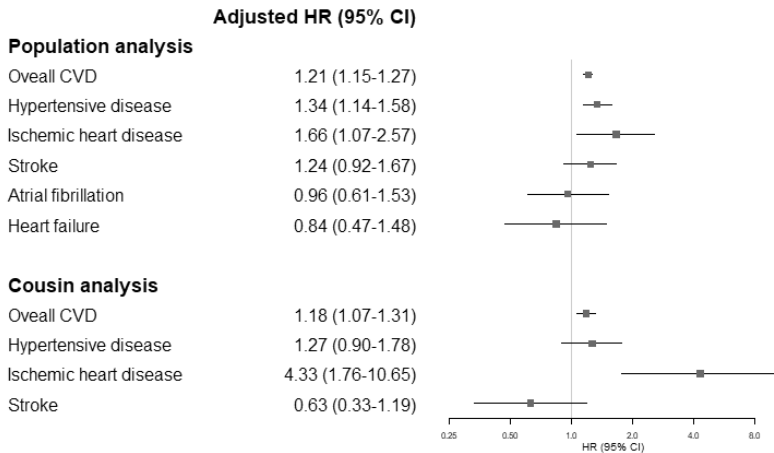
Adjusted HR (95% CI)

Population analysis	
Oveall CVD	1.21 (1.15-1.27)
Hypertensive disease	1.34 (1.14-1.58)
Ischemic heart disease	1.66 (1.07-2.57)
Stroke	1.24 (0.92-1.67)
Atrial fibrillation	0.96 (0.61-1.53)
Heart failure	0.84 (0.47-1.48)
Cousin analysis	
Oveall CVD	1.18 (1.07-1.31)
Hypertensive disease	1.27 (0.90-1.78)
Ischemic heart disease	4.33 (1.76-10.65)
Stroke	0.63 (0.33-1.19)

Figure 5.5. Adjusted hazard ratios and 95% confidence intervals for the association between maternal polycystic ovary syndrome and the offspring's risk of overall cardiovascular disease and its major subtypes

Abbreviations: HR, hazard ratio; CI, confidence interval; CVD, cardiovascular disease. Adjustment was made for sex, country, birth year, and maternal age, country of origin, education, marital status, parity, family history of cardiovascular disease diabetes, psychiatric disorders, and hypertensive disorders prior to the index childbirth.

When examining the joint effect of maternal PCOS and its common comorbidities, we found that offspring of mothers who had both PCOS and conditions such as diabetes, hypertensive disease, or psychiatric disorders had a higher CVD risk than those whose mothers had only PCOS (Figure 5.6).

Maternal conditions	Adjusted HR (95% CI)
Only PCOS	1.21 (1.15-1.27)
Only overweight/obesity	1.01 (1.00-1.03)
Only diabetes	1.21 (1.18-1.25)
Only hypertensive disease	1.21 (1.19-1.23)
Only psychiatric disorders	1.19 (1.17-1.21)
Both PCOS and overweight/obesity	1.20 (1.10-1.30)
Both PCOS and diabetes	1.68 (1.37-2.06)
Both PCOS and hypertensive disease	1.40 (1.17-1.67)
Both PCOS and psychiatric disorders	1.52 (1.29-1.78)

Figure 5.6. Joint effect of maternal polycystic ovary syndrome and other comorbidities before childbirth on the risk of cardiovascular disease in offspring

Abbreviations: HR, hazard ratio; CI, confidence interval; PCOS, polycystic ovary syndrome. Adjustment was made for sex, country, birth year, and maternal age, country of origin, education, marital status, parity, family history of cardiovascular disease, diabetes (except when examining its joint effect with polycystic ovary

syndrome), hypertensive disease (except when examining its joint effect with polycystic ovary syndrome), and psychiatric disorders (except when examining its joint effect with polycystic ovary syndrome) prior to the index childbirth.

5.2.3 Associations between maternal stress in the prenatal period and the risk of HF (Study IV)

In Study IV, 2.5% of study participants were exposed to maternal bereavement the year before or during pregnancy in the prenatal period, and 0.07% had a diagnosis of HF. The incidence rates for HF in the exposed and the unexposed groups were 0.32 and 0.29 per 10 000 person-years, respectively.

Losing any close family member the year before or during pregnancy was not associated with the offspring's risk of HF (Figure 5.7). The association between maternal bereavement in the exposure period and HF in her child did not substantially vary by the time of loss. However, the increased risk of HF was found to be linked to severe forms of bereavement, namely, maternal loss of a partner or older child (adjusted HR: 1.47, 95% CI: 1.06-2.04), as was a loss due to unnatural causes (adjusted HR: 2.77, 95% CI: 1.49-5.17).

When splitting the follow-up at the age of 18 years, the strength of the association between the loss of a partner or an older child and AF in the offspring was stronger in childhood/adolescence (adjusted HR: 2.24, 95% CI: 1.27-3.97) than in adulthood (adjusted HR: 1.25, 95% CI: 0.84-1.86).

Exposure	Adjusted hazard ratio (95% CI)
Any loss	1.04 (0.88-1.23)
Type of deceased relative	
Older child or partner	1.47 (1.06-2.04)
Parent or sibling	0.94 (0.77-1.15)
The deceased's cause of death	
Unnatural cause	2.77 (1.49-5.17)
Natural cause	0.99 (0.83-1.18)
Time of loss	
One year before pregnancy	1.07 (0.87-1.33)
First trimester	1.17 (0.72-1.88)
Second trimester	0.85 (0.53-1.36)
Third trimester	0.99 (0.60-1.66)
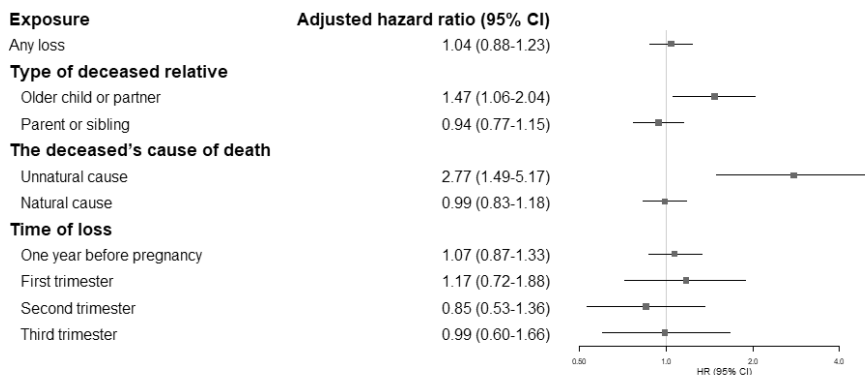
Figure 5.7. Adjusted hazard ratios and 95% confidence intervals for the association between maternal stress the year before or during pregnancy and the risk of heart failure in the offspring

Abbreviations: HR, hazard ratio; CI, confidence interval. We controlled for sex, country, birth year, and maternal age, country of origin, education, marital status, parity, family history of cardiovascular diseases, diabetes, psychiatric disorders, and hypertensive disease prior to the index childbirth. Analyses regarding loss of an older child or partner were restricted to children whose mother had linkage to her partner (N=6,108,486) or at least one live older child (N= 6,031,945) at the baseline, whereas analyses regarding loss of a parent or sibling were restricted to those whose mother had at least a live parent (N=5,614,425) or a live sibling (N=4,437,712) at the baseline.

5.3 Mediating roles of adverse birth outcomes and congenital heart disease in the associations between maternal PCOS and stress with the risk of CVD in offspring

Mediation analyses (Table 5.1) revealed some contributions of congenital heart disease and preterm birth to the associations between maternal conditions, i.e., PCOS and severe stress, and the risk of CVD in offspring. Specifically, congenital heart disease and preterm birth accounted for 10% and 3.9%, respectively, of the association between maternal PCOS and the overall CVD risk in offspring. They accounted for 57.4% and 25.4%, respectively, of the association between severe stress, i.e., death of a partner or older child, and HF risk in offspring. The contribution of abnormal foetal growth to the association of CVD with maternal PCOS or stress appeared to be relatively modest.

Table 5.1. The contribution of preterm birth, abnormal foetal growth, and congenital heart disease in associations between maternal conditions and offspring's CVD risk

Potential mediator	HR (95% CI) [a]			Proportion mediated (%)
	Total effect [b]	Direct effect	Mediated effect	
Association between maternal polycystic ovary syndrome and offspring's overall cardiovascular disease risk				
Preterm birth	1.19 (1.13-1.26)	1.19 (1.13-1.25)	1.01 (1.00-1.01)	3.9
Small for gestational age birth	1.16 (1.10-1.23)	1.16 (1.10-1.24)	0.99 (0.99-1.00)	-
Large for gestational age birth	1.19 (1.13-1.26)	1.19 (1.12-1.26)	1.01 (1.00-1.01)	3.1
Congenital heart disease	1.19 (1.14-1.26)	1.17 (1.12-1.24)	1.02 (1.01-1.02)	10.0
Association between maternal stress and offspring's heart failure risk				
Preterm birth	1.64 (1.16-2.32)	1.48 (1.04-2.11)	1.11 (1.00-1.23)	25.4

Small for gestational age birth	1.64 (1.16-2.31)	1.63 (1.15-2.29)	1.01 (0.99-1.02)	2.2
Congenital heart disease	1.62 (1.15-2.27)	1.26 (0.91-1.75)	1.28 (1.15-1.43)	57.4

Abbreviations: HR, hazard ratio; CI, confidence interval.

[a] We controlled for sex, country, birth year, and maternal age, country of origin, education, marital status, parity, family history of cardiovascular diseases, diabetes, psychiatric disorders, and hypertensive disease prior to the index childbirth.

[b] The estimates for total effect may differ among models corresponding to each mediators because of differences in the number of individuals with missing data in case of each mediator.

6 DISCUSSION

6.1 Summary of the main findings

This thesis, using individual level data from Nordic registers, reveals associations between prenatal risk factors and increased CVD risk up to 48 years of age. Specifically, we found that preterm birth, SGA, and LGA were associated with increased risks of AF (Study I); maternal preeclampsia, particularly its severe forms, was related to increased risks of stroke and IHD in the offspring (Study II); maternal PCOS was associated with increased risks of overall CVD, hypertension, stroke, and IHD in the offspring (Study III); and severe maternal stress, i.e., experiencing the death of a partner or child and the unnatural death of a close family member, was associated with offspring's HF risk (Study IV). After performing sibling or cousin comparison analyses to account for shared familial confounders in Studies I-III, the associations between adverse birth outcomes and the AF risk, maternal preeclampsia and stroke and IHD risk, and maternal PCOS and overall CVD risk generally were attenuated but persisted. Furthermore, our analyses suggest that there is a time-varying association between several prenatal risk factors and CVD outcomes. Most of the observed associations, i.e. between preterm birth or SGA and AF risk, maternal preeclampsia and stroke risk, and severe maternal stress and HF risk, were more strongly associated with the outcome occurring during childhood than in adulthood.

6.2 Interpretation of the main findings

6.2.1 Adverse birth outcomes and the risk of AF (Study I)

Our study contributes to our understanding of the link between preterm birth and increased CVD risk - previously reported mainly for hypertension,[7] IHD,[8] stroke,[9] HF[10] - by showing an association between preterm birth and AF. To our knowledge, only two cohort studies (one conducted in Sweden[70] and one in Finland[71]) investigated this association and both yielded null findings. We speculate that the difference in findings between our and the earlier studies may in part be attributed to survival bias, given the earlier birth years (1914-1952) of participants in these previous studies. Individuals who survived preterm birth under the less advanced neonatal care conditions of that period may constitute a cohort of healthier individuals than those with preterm birth in our cohort.

Our findings also suggest that both SGA and LGA are associated with an increased risk of AF. Similarly, the above-mentioned previous cohort studies observed a 'U'-shaped or a trend towards a 'U'-shaped association between birth weight and AF after adjusting for sex and gestational age. In contrast, two studies have shown linear associations between birth weight and AF. One reported an association between LBW with an increased AF risk,[58] while the other found a link between HBW with AF risk;[67] however, both lacked information on gestational age. To the best of our knowledge, our study is the first to show that excessive foetal growth is associated with the risk of AF later in life. While most existing studies predominantly emphasize the increased CVD risk associated with restricted foetal growth, our findings underscore the importance of exploring the long-term effects of LGA births on cardiovascular health. Given the rising prevalence of LGA births over time, our study highlights the need for further research in this area.

Our finding that the association of preterm birth and SGA with AF risk was stronger in childhood/adolescence than in young adulthood could be attributed to several plausible explanations. First, AF cases may be more likely to be detected in childhood than in adulthood since persons born preterm or with SGA visit the healthcare system frequently during their early years. Second, congenital heart disease is an important risk factor for developing AF in childhood,[172] which in turn is related to preterm birth[173] and SGA.[174] Therefore, the association observed in childhood could be partially explained by congenital heart disease. Third, infants born preterm or with SGA may undergo intensive neonatal care treatments, potentially increasing the risk of AF in childhood.

Given our finding of an association between adverse birth outcomes and AF, it is important to ask whether adverse birth outcomes directly contribute to CVD outcomes or if their effects are attributed to alternative pathways (as illustrated in Figure 6.1). Attributing adverse birth outcomes as the root causes along the causal pathway (Figure 6.1) is challenging, as they are likely to stem from suboptimal intrauterine environments or genetic predispositions. Therefore, from a prevention perspective it is important to understand their determinants. We sought to address these questions in Studies II-IV, and the relevant findings will be discussed in Section 6.2.2.
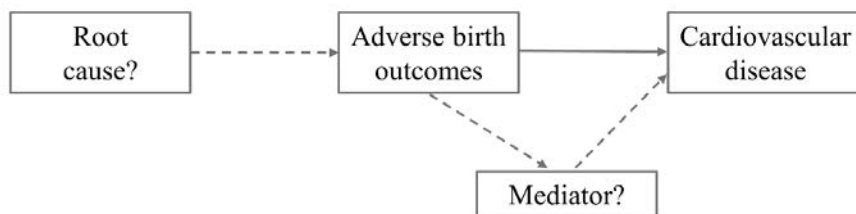
Figure 6.1. Schematic summary for potential causal pathways from prenatal risk factors to the development of cardiovascular disease

6.2.2 Maternal diseases and stress and the risk of CVD in offspring (Studies II-IV)

In Studies II-IV, we analyzed the associations of maternal conditions, namely preeclampsia (Study II), PCOS (Study III), and severe stress (Study IV), with CVD outcomes in the offspring. Additionally, we investigated whether adverse birth outcomes, such as preterm birth, SGA or LGA, and congenital heart disease, could contribute to some of the observed associations.

In Study II, we found increased risks of IHD and stroke among individuals born to mothers with preeclampsia during pregnancy, with the risks being higher in case of severe than in case of mild forms of preeclampsia. This finding aligns with the broader consensus in previous studies that maternal HDP, including gestational hypertension and preeclampsia, are associated with an increased risk of CVD in offspring.[93-96] However, limitations in past research, such as small sample size or insufficient follow-up, have hindered a comprehensive evaluation of the risk of IHD and stroke, two

severe, but rare CVDs in childhood and young adulthood associated with specific forms of preeclampsia.

Severe forms of preeclampsia, including early-onset preeclampsia and preeclampsia with SGA birth, are considered to be placental diseases characterized by vascular lesions, oxidative stress, and perfusion deficits; in contrast, mild forms, including late-onset preeclampsia or preeclampsia without SGA birth, are considered maternal diseases, often occurring in women with constitutional and environmental risk factors, such as multiple births, high BMI and comorbid conditions.[87,175] A recent large Danish cohort study[93] involving over two million live births with 42 years of follow-up observed the highest risk of overall CVD among individuals prenatally exposed to severe or early-onset preeclampsia. However, this study did not explore the associations of preeclampsia subtypes with IHD and stroke, nor did it consider preeclampsia in a pregnancy with SGA birth as a severe form of preeclampsia. Our study extends earlier evidence by showing stronger associations for IHD and stroke in cases of severe compared to mild preeclampsia. This finding further supports the hypothesis that these variants of preeclampsia may represent heterogeneous diseases with potentially differing effects on foetal cardiovascular development.

In Study III, we found that maternal PCOS was associated with increased risks of overall CVD as well as with major CVD types, including IHD and stroke, in offspring from childhood to early middle-age. Importantly, this association was present even in the absence of common maternal comorbidities and assisted reproductive treatment, commonly undertaken by women with PCOS. While a substantial body of evidence suggests that offspring born to women with PCOS are more likely to have cardiometabolic risk factors or preclinical markers for CVD than their unexposed counterparts,[116-118] only two studies have investigated the association between maternal PCOS and CVD risk per se.[119,120] Consistent with our findings, these studies reported increased risks of CVD in offspring exposed to maternal PCOS, albeit with wider confidence intervals than our study due to their limited statistical power. It is important to note that both studies suffered from methodological limitations, including small cohort sizes, short follow-up periods, and insufficient information on confounders, hindering in-depth analyses of the association between maternal PCOS and specific types of CVD.

A significant challenge in this field is disentangling the effect of maternal PCOS from that of its common comorbidities, including obesity, diabetes, hypertensive disease, and psychiatric disorders, all of which have been linked to increased CVD risk in offspring.[93,123,124,127] To address this concern, we investigated the isolated effect of PCOS and explored the combined effects of PCOS with its comorbidities. Our findings revealed that offspring born to mothers with PCOS alone had an increased CVD risk. Notably, when PCOS had comorbidities, the risk of CVD in children was even higher. Further studies are warranted to understand the interplay between maternal PCOS and comorbidities on offspring's CVD risk.

In Study IV, we found no association between prenatal exposure to maternal bereavement and the HF risk up to middle-age. However, individuals born to mothers who experienced more severe forms of bereavement, i.e., the loss of a partner or a child or the unnatural death of a close family member, had increased risks of HF. Evidence regarding the association between prenatal stress exposure and the risk of CVD is limited and inconsistent. Several earlier studies examining prenatal stress exposure used famine and war as stressors,[144-147] however, the survival of individuals through famine and war, characterized by poor nutrition and restricted access to healthcare, may introduce bias by selecting stronger and healthier exposed individuals into the cohort. Only two prior studies in this research field

employed bereavement as a stress exposure. A study conducted by our group[152] found no association between prenatal exposure to maternal bereavement and risks of IHD and stroke. Another study, partly overlapping with our Danish cohort, observed that individuals exposed to maternal bereavement had a modestly increased risk of overall CVD.[151] However, this study did not examine HF as an outcome. Our study is the first to investigate the link between severe maternal stress and the offspring's HF risk. We found that only the most severe forms of stress, such as the loss of a partner or older child or unexpected loss were associated with HF, suggesting the presence of a dose-response association. Though we sought a critical time window for prenatal stress's effect on CVD, our analysis found none. However, the potential for a time-specific effect, particularly in severe stress cases, requires future exploration.

It is noteworthy in Studies II-IV that, despite the rarity of CVD in children, we observed stronger associations of maternal disease or stress with increased CVD risk in childhood than in adulthood. Furthermore, in some instances, the strengths of associations of maternal health conditions and stress with the risk of CVD were stronger compared to those observed in adulthood. Congenital structural or functional heart diseases are more important for pediatric CVD than traditional CVD risk factors such as smoking, obesity, and hypertension which play a major role in the development of CVD in adults.[176] We speculate that the observed associations in childhood may be partly attributed to congenital heart disease, which could, in turn, may be a consequence of adverse *in-utero* environment induced by maternal disease. The results of our mediation analysis indicate that congenital heart disease contributes significantly to the observed association, providing additional support for this explanation.

6.3   Potential underlying mechanisms

Our findings suggest an association between prenatal risk factors, including maternal disease and adverse birth outcomes, and the development of CVD later in life. Two main plausible mechanisms could explain this association: (1) shared familial genetic and environmental factors and (2) foetal programming.

First, evidence suggests that specific genetic variants may be common factors influencing both prenatal risk factors and CVD. For instance, an Australian cohort study identified four genetic variants associated with maternal preeclampsia and the offspring's CVD risk factors.[103] Similarly, shared environmental risk factors within families, such as unhealthy lifestyle habits, low educational attainment, low socioeconomic status, or exposure to pollution, might be linked to both maternal characteristics and the offspring's cardiovascular health. To investigate the role of shared familial factors, we employed a family-based study design, using sibling (for Studies I and II) and cousin comparison (for Study III). The results from our sibling analyses and cousin analyses showed that most of the observed associations between prenatal exposures and CVD outcomes attenuated but persisted, suggesting that genetic predisposition and familial environmental risk factors may partly, but not entirely, explain our findings.

Second, maternal conditions such as preeclampsia and PCOS, or severe stress during pregnancy may contribute to a suboptimal intrauterine environment characterized by oxidative stress, inflammation, placental insufficiency, and abnormal hormone levels, which, in turn, can lead to adverse changes in the immune system, the renin angiotensin aldosterone system, the HPA axis, the sympathetic nervous

system, and to epigenetic modifications.[37,101,153,177] All these pathophysiological alterations could further program foetal development, leading to prematurity, abnormal foetal growth, cardiovascular remodelling and dysfunction. Ultimately, these changes increase the risk of developing hypertension, IHD, stroke, AF, HF, or other forms of CVD later in life. In support of this hypothetical mechanism, results from our mediation analyses suggested that congenital heart disease, preterm birth, and restricted foetal growth may be involved in the foetal programming of CVD.

It is important to note that we chose not to employ mediation analysis to investigate the roles of preterm birth, foetal restriction, and congenital heart disease in the association between maternal preeclampsia and offspring's CVD. This decision was made due to the complexity of preeclampsia. Early-onset preeclampsia is often accompanied by preterm delivery or foetal growth restriction, but it is not always clear whether preeclampsia causes the latter two conditions or if all three conditions occur simultaneously. Nevertheless, we observed a persistent association between maternal preeclampsia and CVD in a subgroup comprising only those with preeclampsia, excluding preterm birth, SGA, or congenital heart disease (data not shown), suggesting the existence of alternative pathways beyond these three factors.

6.4 Methodological considerations

6.4.1 Confounding

A primary challenge in all observational studies striving to address etiological research questions and establish reliable causal inferences is confounding. If a third variable could, at least in part, explain the association between prenatal risk factors and CVD outcomes without being a consequences of any of them in our studies, then confounding exists, and the third variable is considered a confounder. We selected potential confounders by including a set of available covariates that are associated with both the studied prenatal risk factors and CVD outcomes, excluding those on the causal pathway between them. We addressed confounding by adjusting for these potential confounders in multivariable models or a family-based study design to control for the unmeasured familial confounders. However, we should note that the assumption of the absence of confounding in observational studies is too strong to be held, and residual confounding cannot be entirely ruled out from our investigations.

6.4.2 Nordic registry-based research: strengths and limitations with a special focus on selection and information bias

The Nordic countries feature an extensive array of government-maintained registries designed for administrative and research purposes. The Nordic registry data is widely deemed a 'gold mine' for epidemiological research[178] due to its vast potential, providing researchers invaluable individual-level data spanning various aspects of human life. Compared with traditional cohort studies, our register-based cohort possesses several unique strengths:

- The administrative data often have higher validity and completeness than self-reported data.
- We used the entire countries rather than sampling data within the population, thereby reducing selection bias to a significant extent.

- The substantial sample size and lifelong follow-up (with censoring often only at emigration or death) enabled us to investigate associations between rare exposure and rare outcomes.

However, registry-based cohort studies, including ours, are not immune to misclassification of the exposure, outcome, or confounders. Despite validation studies showing high positive predictive values for maternal diseases such as preeclampsia and PCOS, as well as children's CVD outcomes like IHD, stroke, AF, and HF diagnoses in Nordic patient registries,[159-164,179-181] it is essential to note that milder cases of several of these diseases may not be captured. Besides, there is a possibility of differential misclassification. In case of detection bias, individuals born preterm, with low birth weight, or to mothers with severe health conditions may seek medical attention more frequently than those born after a normal pregnancy. This increased interaction with health services could lead to a higher likelihood of diagnosing CVD outcomes, which may somewhat overestimate the association of prenatal risk factors with CVD. In addition to exposure and outcome misclassification, the misclassification of confounders during measurement could affect the effect of the adjustment for these factors.

6.4.3 Sibling comparison design: potential advantages and challenges

Studies I and II included a sibling comparison design to control for unmeasured familial confounding factors. This design involves establishing a sub-cohort consisting of sibling pairs within the entire cohort. By running Cox regression with a family stratum, only sibling pairs discordant for exposure and outcome contribute to the risk estimates. Subsequently, the associations observed from this sibling sub-cohort could be free of confounders shared among siblings, such as genetic traits, family socioeconomic status, lifestyle factors, and neighborhood influences. If the association observed in the entire cohort decreases in the sibling design, it may suggest the presence of familial confounding.

While the sibling comparison design offers notable strengths in controlling for familial confounders, it is imperative to acknowledge certain limitations inherent to this approach. First, the sibling design is sensitive to the number of available sibling pairs, potentially limiting statistical power and generalizability. Second, this design not only automatically controls for all confounders shared within families but also controls for shared mediators, complicating the interpretation of whether the attenuation of the association is attributable to familial confounders or shared mediators. Third, the design may amplify measurement error and confounding by non-shared factors that may influence both the exposure and outcome.[182] Fourth, this design is susceptible to bias if a carryover effect exists,[183] wherein the first pregnancy's exposure and outcome influence subsequent pregnancies. Nevertheless, these limitations should not be exaggerated, as they may also be present in the entire cohort study. Improving statistical power, adjusting for non-shared confounders, and minimizing measurement error are strategies that could alleviate some of these limitations.

7 CONCLUSIONS

This thesis investigated the association between prenatal risk factors and the development of CVD up to early middle-age. The key conclusions drawn from the main findings of the four studies incorporated in this thesis are:

- The increased AF risk associated with preterm birth and excessive foetal growth (using LGA as a proxy) persisted from childhood into adulthood, while the association with restricted foetal growth (using SGA as a proxy) was observed only in childhood.
- Maternal preeclampsia during pregnancy, particularly its severe forms, including preeclampsia with SGA and ealy-onset preeclampsia, was associated with heightened risks of IHD and stroke in offspring.
- Maternal PCOS was associated with offspring's increased risks of overall CVD, hypertension, IHD, and stroke. There was some modest evidence that the association between maternal PCOS and the overall CVD risk would be mediated by congenital heart disease, SGA, and preterm birth.
- Severe maternal stress, namely, the bereavement resulting from the death of a partner or child or unnatural death of a close family member, was related to an increased risk of HF. Congenital heart disease and preterm birth substantially contributed to this association.
- Several of the observed associations, namely adverse birth outcomes and the AF risk, maternal preeclampsia and stroke and IHD risk, and maternal PCOS and overall CVD risk, were attenuated but persisted after sibling or cousin comparison analyses, suggesting that genetic or environmental risk factors shared within families could not fully account for the observed associations.
- Our findings suggested a time-varying association between prenatal risk factors and CVD outcomes, with several associations being stronger during childhood than during adulthood.

In summary, this thesis suggests the potential role of prenatal risk factors for long-term cardiovascular health. It emphasizes the need for continued investigation into the complex interplay between early-life factors and cardiovascular outcomes in order to be able to develop targeted interventions.

8 POINTS OF PERSPECTIVE

After finding the presented associations between adverse birth outcomes, maternal diseases, or severe maternal stress and the risk of CVD up to early middle-age, several critical questions emerge, necessitating further exploration in future research.

The primary and most intriguing question is establishing causality in the observed associations. While a randomized clinical trial is the gold standard for causal inference, conducting such a trial among pregnant women is ethically and practically unfeasible. An alternative approach is Mendelian randomization, an increasingly employed design in cardiovascular research. Future studies with genetic and clinical data should consider employing this method to establish a causal association between prenatal risk factors and CVD risk methodology. However, careful attention should be given to meeting the assumptions underlying this approach.

The second unresolved question is to understand how maternal diseases, such as preeclampsia, PCOS, diabetes, and obesity, or severe maternal stress influence the cardiovascular development of their offspring. Although our causal mediation analyses suggest potential pathways involving prematurity, foetal growth restriction, or abnormal cardiac vascular structure and function, comprehensive explanations for the observed associations require further epidemiological and experimental studies. Unraveling these underlying mechanisms could pave the way for targeted prevention strategies for individuals at high risk of CVD.

Taking a life course perspective, risk factors at various stages of life - during foetal life, infancy, childhood, young adulthood, and mid-adulthood - have the potential to influence CVD later in life. Well-established risk factors, including smoking, unhealthy eating, physical inactivity, and obesity, continue to play a pivotal role in the development of CVD. Therefore, the third critical question requiring examination is the interaction between risk factors during the prenatal period and later stages in relation to the risk of developing CVD.

Given that our studies only followed individuals up to early middle-age, it remains unclear how these associations translate to most CVD cases, i.e., to those commonly seen in the elderly. This warrants investigation in future studies when relevant data becomes available in the Nordic health registers.

If future research can establish causal associations, early-life preventive measures may offer a novel approach for policies and health programs to alleviate the burden of CVD. Acting within the narrow time window before childbirth might mitigate adverse epigenetic changes that predispose individuals to CVD, potentially providing more significant health benefits than prevention programs implemented later in life when atherosclerosis is already advanced. Just as a Chinese proverb says, "The excellent doctor works to prevent sickness; the mediocre doctor addresses impending sickness," the earlier the prevention of cardiovascular diseases, the greater the benefits it may yield.

9 ACKNOWLEDGEMENTS

Writing this final chapter proved to be one of my most difficult writing endeavors. How can I convey my gratitude to the fantastic individuals I encountered throughout the four-year journey of my Ph.D. within the space constraints of this section and with concise language? Nonetheless, I would like to seize this moment and extend my heartfelt thanks to all those who have nurtured, assisted, supported, warmed, and enlightened me during this significant phase of my life. Despite the high risk of inadvertently leaving someone out, I wish to express my gratitude to:

My main supervisor, **Krisztina László**. Thank you for providing me with such a compelling and meaningful project and that has fueled my curiosity and dedication to understanding the "why." I take immense pride in sharing with those around me that, as a Ph.D. student at Karolinska Institutet, I am exceptionally fortunate to have the world's most responsible, reliable, supportive, and inspiring supervisor. Your qualifications fulfill my expectations of being a great role model. I have gained valuable insights not only in our research domain but also in scientific thinking and meticulous work practices. Your guidance has been invaluable, and I am confident that I can always count on your support when facing challenges in both work and life.

My co-supervisor, **Imre Janszky**. I wholeheartedly agree with Krisztina's statement that you are a guru in epidemiology and cardiovascular research. Your remarkably profound knowledge never fails to impress me. I am grateful for your active involvement in my project, for providing prompt responses, and for your encouragement and appreciation for even the most minor achievements I made. Your unwavering support and care have made me feel both supported and confident. Thank you for contributing to my growth and helping me become a better version of myself.

My co-supervisor, **Jiong Li**. I owe the realization of my educational aspirations in a foreign setting mainly to you. Thank you for transforming my dream into reality. Even before arriving in Sweden, you opened my mind to new perspectives. Through you, I learned the beauty of Nordic culture, and you inspired me to embrace change. Your significant intellectual contributions and consistent support as the responsible coordinator in our collaborative project have been instrumental in the progress of my work. My project wouldn't have been possible without your guidance and support.

My co-supervisor, **Nathalie Roos**. I am very grateful to have you on my supervisory team. Your kindness, willingness to generously share your expertise, approachability, and helpful nature have significantly impacted on my academic journey. I appreciate the opportunity to participate in your group's bi-weekly seminars, as they have greatly enhanced my understanding of the clinical focus. Thank you for your invaluable support.

My mentor, **Kyriaki Kosidou**. I am fortunate to have a mentor as wise as you. You serve not only as a guide but also as a philosophical and spiritual mentor. Your ability to interpret challenges in my work and life from a philosophical perspective has provided me with a unique and insightful lens through which to view situations. Your encouragement to not set limitations on my future and to consider options beyond academia has opened my mind to new possibilities. Your exceptional mentoring has played a crucial role in my career development, and I am truly grateful for your guidance.

们这个家付出的一切，"执子之手与子偕老"是我对咱俩最大的期盼。最后我要感谢一个名叫 Leo（高乐扬）的神奇的小男孩，小小的身躯里有颗大大的爱心，你对我的爱可以融化斯德哥尔摩最寒冷的雪，做你的妈妈是最最幸福的一件事，谢谢你的存在让我的世界变得更加美好！

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