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Genetic epidemiological studies of adverse pregnancy outcomes and the role of schizophrenia

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Stockholm 2006

Published and printed by Karolinska University Press
Box 200, SE-171 77 Stockholm, Sweden

ISBN 91-7140-590-9

SUMMARY

The purposes of this thesis were to investigate the importance of genetic and environmental factors in the development of adverse pregnancy outcomes and to investigate the role of schizophrenia as a risk factor for and a consequence of adverse pregnancy outcomes.

In the first study we investigated the importance of genetic and environmental factors for pre-eclampsia and gestational hypertension and whether the diseases share genetic etiology. Swedish population-based registers were used and 1,188,207 births were included. We found that full sisters and mother-daughters were more similar for pre-eclampsia and gestational hypertension than half-sisters. Moreover, a full sister to a woman with pre-eclampsia had a significantly increased risk of gestational hypertension. The heritability estimates were 31% for pre-eclampsia, 20% for gestational hypertension, and 28% for pregnancy-induced hypertension. In sum, we found a genetic component in the liability of both pre-eclampsia and gestational hypertension. The co-morbidity indicates that pre-eclampsia and gestational hypertension may share parts of their genetic etiology.

The aims of the second and third studies were to examine risks of adverse pregnancy outcomes among mothers and fathers with schizophrenia and to investigate if the increased risks among women with schizophrenia were due to maternal, paternal, genetic and/or environmental factors. Two million births from Swedish population-based registers were included. Maternal factors (e.g., smoking) explained most of the risks for adverse pregnancy outcomes and women with an episode of schizophrenia during pregnancy had the highest risks. Increased risks for low birth weight, small-for-gestational age, and infant death among offspring to fathers with schizophrenia were observed. In conclusion, mothers with schizophrenia have increased risks for adverse pregnancy outcomes. The risks were highest for women admitted to psychiatric care during pregnancy. The increased risks among offspring to fathers with schizophrenia suggest that, in addition to maternal risk behavior, non-optimal social and/or economical circumstances are of importance.

In the fourth study we investigated if the previously found association between low birth weight and subsequent development of schizophrenia is mediated by familial factors. We used data from obstetric records in a cohort analysis of 11,360 same-sexed twins, and conducted co-twin control analyses on 90 pairs discordant for schizophrenia. The results from the cohort analyses showed that low birth weight and small head circumference were associated with later development of schizophrenia. The associations remained in the within-pair analyses. We concluded that the association between low birth weight and schizophrenia is partly a function of reduced fetal growth and that fetal growth restriction seems to be associated with risk of schizophrenia independently of familial factors.

This thesis has examined adverse pregnancy outcomes using register-based samples and genetic epidemiological methods. We found a genetic comorbidity between pre-eclampsia and gestational hypertension, which should be considered in the future search for susceptibility genes and in the identification of intervention strategies. Maternal as well as paternal schizophrenia influence the risk of adverse pregnancy outcomes. These results prompt for better surveillance of families at risk. Further, we found that fetal growth restriction is an independent risk factor for subsequent development of schizophrenia. Adverse pregnancy outcomes represent some of the most challenging targets in epidemiology and elucidation of the underlying mechanisms and identification of markers of early insult that predisposes to adult diseases are important.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-IV):

I. Nilsson E, Salonen Ros H, Cnattingius S & Lichtenstein P.
The importance of genetic and environmental effects for pre-eclampsia and gestational hypertension: a family study.
BJOG, 2004, 111, 200-206.

II. Nilsson E, Lichtenstein P, Cnattingius S, Murray RM & Hultman CM.
Women with schizophrenia: pregnancy outcome and infant death among their offspring.
Schizophrenia Research, 2002, 58, 221-229.

III. Nilsson E, Hultman CM, Cnattingius S, Otterblad Olausson P & Lichtenstein P.
Schizophrenia and adverse pregnancy outcomes – Maternal, paternal and genetic influences.
Submitted.

IV. Nilsson E, Stålberg G, Lichtenstein P, Cnattingius S, Otterblad Olausson P & Hultman CM.
Fetal growth restriction and schizophrenia: a Swedish twin study.
Twin Research and Human Genetics, 2005, 8, 402-408.

Previously published papers were reprinted with kind permission from Blackwell Publishing (paper I), Elsevier (paper II) and Australian Academic Press (paper IV).

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LIST OF ABBREVIATIONS

c_c^2	Shared environment by the entire family
c_s^2	Shared sister environment
CI	Confidence Interval
DSM	Diagnostic and Statistical Manual of Mental Disorder
DZ	Dizygotic
e^2	Non-shared environment
GEE	Generalized Estimation Equation
GH	Gestational hypertension
h^2	Heritability
ICD	International Classification of Diseases
MZ	Monozygotic
OR	Odds Ratio
PIH	Pregnancy-induced hypertension
SD	Standard deviations
SGA	Small-for-gestational-age
SIDS	Sudden Infant Death Syndrome

INTRODUCTION

Complications during pregnancy and delivery are closely related to perinatal mortality and morbidity as well as maternal mortality. Low socio-economic status and maternal smoking are examples of identified environmental risk factors for adverse pregnancy outcomes. Moreover, studies have shown familial aggregation and therefore the relative importance of genetic and environmental components should be investigated.

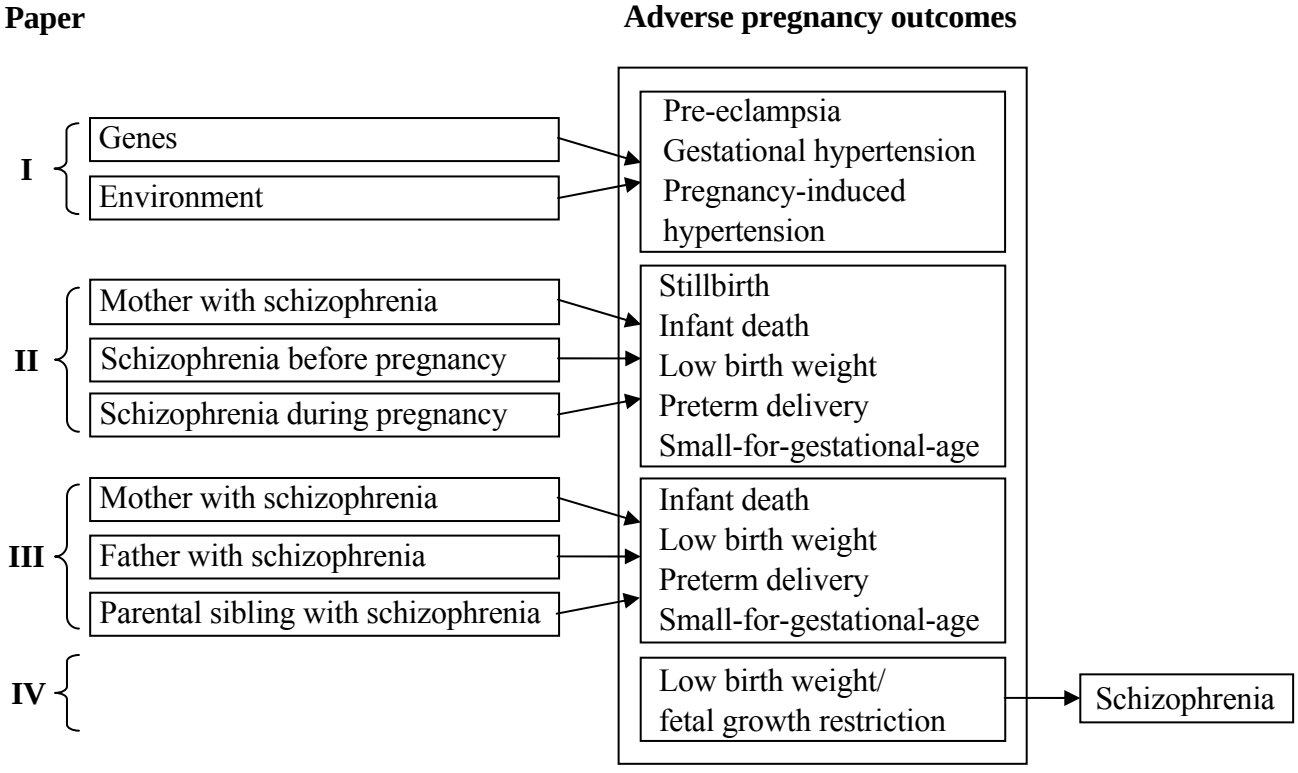
There is also a need to address the role of chronic maternal disorders, maternal life-style and social disadvantage for adverse pregnancy outcomes. Women with chronic maternal disease face an increased risk of poor pregnancy outcome and elucidation of the mechanisms underlying these associations is crucial. Studies have shown that maternal schizophrenia is a risk factor for complications during pregnancy and delivery and it is of interest to investigate if paternal schizophrenia also increases the offspring's risk for obstetric complications or if the associations could be explained by maternal behaviour and/or familial factors.

Obstetric complications are also increasingly considered to represent important risk factors associated with the later development of both somatic and psychiatric diseases in the offspring. Over the past decade, data from numerous epidemiological studies have indicated inverse associations between birth weight and risks of coronary heart disease, hypertension, type 2-diabetes, and schizophrenia in adulthood (Barker, 2002; Hultman *et al.*, 1999; Sallout & Walker, 2003). Elucidation of the types of adverse factors involved, and whether their influences are independent of genetic predisposition, could help to trace the mechanisms by which such factors increase risk for subsequent disorder.

This work will focus on the influence of adverse pregnancy outcomes, both as an outcome and as a risk factor for disease (Figure 1). First in paper I, we investigated the importance of genetic and environmental influences in the etiology on two major pregnancy complications, pre-eclampsia and gestational hypertension. Thereafter, in paper II, we set out to investigate the importance of maternal schizophrenia for adverse pregnancy outcomes. Specifically, we investigated if women with schizophrenia were at increased risks for stillbirth, infant death, preterm delivery or giving birth to low-birth-weight or small-for-gestational-age infants. Important confounding factors known

to be more prevalent among mothers with schizophrenia, such as maternal smoking and single motherhood were included. Further, we investigated whether the risks for adverse pregnancy outcomes were higher if the mother was admitted to hospital for schizophrenia during pregnancy. In paper III, we investigated the mechanism underlying the association between maternal schizophrenia and risks for adverse pregnancy outcomes. We wanted to answer questions such as: Are the associations due to maternal and/or paternal factors? Are the associations explained by genetic and/or environmental factors? In paper IV, the consequences of adverse pregnancy outcomes were addressed. We studied whether there was an association between fetal growth restriction and subsequent development of schizophrenia and also whether low birth weight and intrauterine growth restriction were directly related to adult schizophrenia or whether this association could be due to familial factors.

Figure 1. Schematic overview of the thesis.



BACKGROUND

ADVERSE PREGNANCY OUTCOMES

Pregnancy-induced hypertensive diseases

Seizure in pregnancy or during delivery has been a feared pregnancy complication for more than two thousand years. In the mid 1800s, researchers found that protein in the urine antedated the seizures (Roberts & Lain, 2002). It was soon recognized that the syndrome, even without seizures (pre-eclampsia) presented risks to mother and baby. The etiology of pre-eclampsia and eclampsia is yet unknown and pre-eclampsia has long been regarded as a pregnancy-induced hypertensive disease. In this work we have studied pre-eclampsia and gestational hypertension defined according to the ICD classifications and reported to the Medical Birth Register. The disorders develop in 2-10 per cent of all pregnancies (Duckitt & Harrington, 2005; Ros *et al.*, 1998), and identified risk factors include for example; a history of pre-eclampsia, pre-existing diabetes, multiple pregnancy, family history, nulliparity, a raised body mass index before pregnancy, and hypertension (Duckitt & Harrington, 2005).

Low birth weight

Low birth weight is defined as a birth weight below 2,500 grams and 4.3 per cent of all infants born in Sweden in 2003 had low birth weight. Birth weight is closely associated with perinatal mortality and morbidity, and also with risks of chronic diseases later in life. Studies on birth weight has long been a focus for clinical and epidemiological studies (Kramer, 1987). According to the 'Fetal origins hypothesis' or the 'Barker hypothesis', undernutrition in utero permanently changes the body's structure, physiology, and metabolism, and leads to coronary heart disease and stroke in adult life (Barker, 1998). Diseases like breast cancer and schizophrenia have also been proposed to have its origin in fetal nutrition (Ozanne *et al.*, 2004). Studies on risk factors for low birth weight have given mixed results. An explanation for this may be that birth weight is determined by two components; the duration of gestation and the intrauterine growth rate. If the causal determinants for these two factors differ it is likely that studies of different populations give varying results (Kramer, 1987). Both genetic and environmental components are of importance in the etiology of low birth weight. The correlation in birth weight for siblings has been estimated to 0.5, and maternal and paternal birth weights have been found to correlate with offspring birth weight (Magnus *et al.*, 2001). Specific genetic variants influencing birth weight have also been

identified including variation in the gene for insuline-like growth factor 1 (Randhawa & Cohen, 2005). Birth weight is influenced by a number of maternal health-, anthropometric-, and socio-demographic characteristics including maternal smoking during pregnancy, maternal weight and height, age, ethnicity, parity, and socio-economic status (Kramer, 1987).

Preterm delivery

Preterm delivery is defined as the delivery of an infant before 37 weeks of gestation. According to the Medical Birth Register, 5.7 per cent of all deliveries in Sweden in 2003 were preterm. Preterm birth is a major public health concern, and most neonatal mortality and morbidity may be attributed to preterm birth (Wen *et al.*, 2004). Preterm born infants are also at increased risks for long term consequences, such as neurodevelopmental handicaps, chronic respiratory problems and visual impairment (Berkowitz & Papiernik, 1993). Several risk factors for preterm delivery have been identified, including prior preterm delivery, low body weight, younger maternal age, low socio-economic status, and maternal smoking. Other important factors associated with preterm delivery are, for example, multiple pregnancy, infections, preterm premature rupture of membranes, pre-eclampsia, and chorioamnionitis (Berkowitz & Papiernik, 1993).

Small-for-gestational-age

Small-for-gestational-age (SGA) is in the Medical Birth Register generally defined as birth weight more than 2 SDs below the mean estimated fetal weight for gestational age according to the Swedish standard curve (Marsal *et al.*, 1996). The proportion of small-for-gestational-age births in Sweden in 2003 was 2.3 per cent. Several risk factors have been identified, for example pre-pregnancy weight, maternal height, maternal and paternal birth weight, paternal height and weight, primiparity, maternal smoking, pre-eclampsia, and socio-economic status (Kramer, 1987). Also, the familial aggregation of small-for-gestational-age births is substantial (Jaquet *et al.*, 2005). SGA is commonly used as a proxy for intrauterine growth restriction. Fetal growth restriction is associated with intrauterine asphyxia, which may lead to fetal death, neonatal or long term morbidity (Kramer, 1987).

Stillbirth

Stillbirth accounts for more than a third of all fetal and infant deaths and for about half of the perinatal deaths in Europe and North America (Goldenberg *et al.*, 2004). In the Swedish Medical Birth Register, and subsequently in this work, stillbirth is defined as fetal death at 28 or more completed weeks of gestation. Since the beginning of the 20th century the rates of stillbirths has decreased substantially in Sweden. However, during the last 20 years the rate of stillbirths has been fairly stable and was in 2003 3.4 per 1,000 births. Several factors have been identified as causes of stillbirth, for example, severe congenital malformations, maternal/fetal infection, placental complications such as placental abruption and placentae praevia, and fetal growth restriction (Goldenberg *et al.*, 2004). Risk factors associated with stillbirth include; prior stillbirths, low socio-economic status, high maternal age, diabetes mellitus, maternal overweight, and smoking (Stephansson *et al.*, 2001).

Infant death

The rate of infant death has decreased substantially during the last century and has, in contrast to the rate for stillbirths, continued to decrease during the last decades. In 2003, 3.1 infant per 1,000 births died within the first year of life. The continued decline of infant deaths has primarily been attributed to improvements in neonatal care. The major causes of the neonatal deaths (i.e., deaths during the four first weeks of life) are immaturity and congenital malformations. Infections, cerebral hemorrhage, and respiratory illness are also possible causes (Luginaah *et al.*, 1999). The risk factor pattern for infant death is similar to that of stillbirth and includes smoking, low socio-economic status, and multiple births (Luginaah *et al.*, 1999). An inverse association between socioeconomic status and risk of postneonatal mortality has been shown. However, it is not clear whether this association is due to confounding by maternal smoking, low maternal age, or high parity (Arntzen *et al.*, 2004; Nordstrom *et al.*, 1993).

RISK FACTORS FOR ADVERSE PREGNANCY OUTCOMES

Genetic and environmental influences on pre-eclampsia and gestational hypertension

Pregnancy-induced hypertensive diseases are associated with both maternal mortality and increased risks of perinatal mortality and infant morbidity (Cnattingius *et al.*, 1997). Pre-eclampsia and gestational hypertension develop in 2-10 per cent of all pregnancies. The incidence varies depending on the population studied and definition of the disorders (Duckitt & Harrington, 2005; Ros *et al.*, 1998). Pre-eclampsia is associated with adverse outcomes such as intrauterine growth restriction and preterm birth (Chesley, 1984). A recent systematic review identified a number of risk factors associated with pre-eclampsia, for example, a history of pre-eclampsia, pre-existing diabetes, multiple pregnancy, family history, nulliparity, a raised body mass index before pregnancy, high maternal age, renal disease, hypertension, more than 10 years since the last pregnancy, and raised blood pressure at the first visit to antenatal care (Duckitt & Harrington, 2005). Although gestational hypertension is often considered a less severe condition (Naeye, 1981; Page & Christianson, 1976), the risks for adverse perinatal outcomes are higher in severe gestational hypertension than in mild pre-eclampsia (Buchbinder *et al.*, 2002). The primary aetiology of both conditions remains essentially unknown (Roberts & Cooper, 2001a). Despite advances in perinatal care, the incidence of pre-eclampsia has not changed (Sibai *et al.*, 2005) and the present treatment to reverse the syndrome has for the last 100 years been delivery (Roberts & Lain, 2002).

A recent systematic review found that family history of pre-eclampsia almost tripled the risk of pre-eclampsia (Duckitt & Harrington, 2005), and maternal pre-eclampsia has been shown to be associated with 70 per cent excess risk of pre-eclampsia in daughters (Mogren *et al.*, 1999). Results from other studies also support the hypothesis of a familial susceptibility to pre-eclampsia (Carr *et al.*, 2005; Redman & Sargent, 2005; Salonen Ros *et al.*, 2000; Skjaerven *et al.*, 2005). A maternal genetic component of the liability of developing pre-ecampsia has been identified, and several models of inheritance have been proposed (Arngrimsson *et al.*, 1995; Cooper & Liston, 1979; Sutherland *et al.*, 1981). There is also a genetic susceptibility to gestational hypertension, although probably weaker than that for pre-eclampsia (Salonen Ros *et al.*, 2000).

Even though the existence of a genetic influence in the development of pre-eclampsia is evident, the magnitude of the genetic influence of pre-eclampsia remains largely unknown. The reason is that genetic influences are ordinarily estimated from twin studies, comparing co-morbidity in monozygotic twins and dizygotic twins. Due to rarity of the disease there is, as far as we know, only one twin study with sufficient sample size to estimate the heritability for liability to pre-ecampsia and gestational hypertension (Salonen Ros *et al.*, 2000). Two other studies using hospital records, could not find any concordant pairs (Thadhani *et al.*, 1999; Treloar *et al.*, 2001).

Previous studies have found that women who develop pre-eclampsia in their first pregnancy have an increased risk for gestational hypertension in their second pregnancy (Campbell *et al.*, 1985). Moreover, the risk factor pattern for pre-eclampsia and gestational hypertension has been shown to be similar. Ros et al. (1998) found remarkable similarities in risk factor patterns for pre-eclampsia and gestational hypertension, although the magnitude of the observed associations often differed between the two conditions. Increased pre-pregnancy body mass index was associated with similar increases in risks for both conditions, whereas smoking reduced the risks of both conditions. These findings indicate a shared etiology for the development of these conditions (Ros *et al.*, 1998), and indicates that pre-eclampsia and gestational hypertension are different expressions of the same genetic and environmental liability.

Schizophrenia as a risk factor for adverse pregnancy outcomes

Chronic maternal diseases are commonly associated with increased risks of adverse pregnancy outcomes, including infant mortality, morbidity, and neurodevelopmental impairment. One of these diseases is schizophrenia. There is evidence that mothers with a diagnosis of schizophrenia have increased risks for complications during pregnancy and delivery (Sacker *et al.*, 1996). The notion of schizophrenia as a highly heritable disease has captured specific attention to the fetal environment of high risk offspring. Some of the genes predisposing to schizophrenia may act via early developmental effects, with adverse pregnancy outcomes representing part of the longitudinal phenotype of schizophrenia.

During the last years, large population-based register studies on schizophrenic mothers' risk for adverse pregnancy outcomes have been presented (Bennedsen *et al.*, 1999; Jablensky *et al.*, 2005; Webb *et al.*, 2005). A Danish study reported that women with

schizophrenia were at increased risk for preterm delivery and of giving birth to low birth-weight or SGA infants (Bennedsen *et al.*, 1999). Moreover, similar results have been reported from other population-based studies (Jablensky *et al.*, 2005; Webb *et al.*, 2005). However, one meta-analysis and one review of studies conducted before 1990 showed conflicting results. McNeil et al. (2001) concluded that the incidence of somatic complications during pregnancy and delivery in schizophrenic women was not increased, although Sacker et al. (1996) found a small but statistically significant increased risk for adverse pregnancy outcomes among women with schizophrenia. The methodological difficulties of these earlier studies are evident. The conflicting results might be due to small sample size. The relatively low frequency of adverse pregnancy outcomes and schizophrenia demands large samples to have sufficient precision to reliably quantify risks of adverse pregnancy outcomes among women with schizophrenia (Byrne *et al.*, 2000). The different definitions of schizophrenia could also explain the disparate results (Bennedsen, 1998; McNeil, 1991; Sacker *et al.*, 1996). Moreover, some studies are based on maternal recall of obstetric complications. Finally, although the more recent population-based studies have sufficient power to detect an effect, they have not been able to take important confounding factors into account.

The mechanisms underlying the increased risk of adverse pregnancy outcomes related to maternal schizophrenia is still not clear. First, maternal behavior during pregnancy such as smoking, use of illicit drugs, pharmacological treatment or socio-economic conditions may explain the increased risks for adverse pregnancy outcomes among women with schizophrenia. Many studies have found a significantly higher prevalence of smoking among individuals with schizophrenia than among other groups (de Leon *et al.*, 1995; Diwan *et al.*, 1998; Goff *et al.*, 1992; McNeil *et al.*, 1983). Smoking is a well-known risk factor for adverse pregnancy outcomes and causally related to fetal growth restriction (Kramer, 1987; Sexton & Hebel, 1984). However, it is not investigated whether these smoking and socio-economic differences are responsible for the increased risks for adverse pregnancy outcomes among schizophrenic women. Second, fathers may also contribute to the association. The risk for obstetric complications in offspring to fathers with schizophrenia is mainly unknown. However, it is likely that paternal schizophrenia creates non-optimal social and/or economical living conditions in the family. Third, the association could be explained by genetic factors. Exposure to early environmental risk factors, such as pre- and perinatal

complications, increases the vulnerability for schizophrenia (Cannon *et al.*, 2002), which is to be expected if a common genetic factor for adverse pregnancy outcomes and schizophrenia exist. Even though the neurodevelopmental hypothesis suggest a causal effect between early environmental factors and risk of schizophrenia, epidemiological studies have not yet convincingly excluded the possibility of genetic confounding.

CONSEQUENCES OF ADVERSE PREGNANCY OUTCOMES

The number of adult diseases suggested to have part of their origin in the course of adverse pregnancy outcomes (particularly low birth weight and fetal growth restriction) grows steadily. It includes amongst others; cardiovascular disease, hypertension, stroke, depression, and schizophrenia. Based on the findings of an increased risk for adverse pregnancy outcomes among mothers with schizophrenia and studies showing an increased risk for low-birth-weight infants to develop schizophrenia in adulthood (Cannon *et al.*, 2002), it is of importance to elucidate whether these associations could be confounded by familial (primarily genetic) factors.

Schizophrenia affects about 1 per cent of the population and similar rates have been observed across different countries and cultural groups. The illness is among the world's top ten causes of long-term disability and the symptoms include severe disturbances in the process of thought and perception, apathy, withdrawal and cognitive impairment (Mueser & McGurk, 2004). Both genetic and environmental factors appear to be of importance in the etiology of schizophrenia. A first-degree relative to a patient with schizophrenia has a tenfold increased risk of developing the disease compared to individuals without schizophrenia in the family (Mueser & McGurk, 2004). A quantitative summary of the results from twin studies of schizophrenia showed a high heritability (81%, 95% CI 73%-90%) and a small but significant shared environmental component (11%, 95% CI 3%-19%) (Sullivan *et al.*, 2003). The presence of a significant common environment suggests that these effects most likely occur before and around birth because the environment for twin pairs is most similar in utero and in the immediate postnatal period (Fish & Kendler, 2005). The results of a shared environmental component in the etiology of schizophrenia supports the hypothesis of schizophrenia as a neurodevelopmental disorder originating in early pregnancy (Sullivan *et al.*, 2003). The 'neurodevelopmental hypothesis' of schizophrenia became prominent during the 1980s and postulates that genetic factors involved in

neurodevelopment and/or environmental insults in early life lead to aberrant brain development, which in turn predisposes to the later onset of schizophrenia (Broome *et al.*, 2005; Weinberger, 1987). Further evidence for early developmental aberrations in the development of schizophrenia is derived from studies showing that individuals who later develop schizophrenia are more likely than healthy comparison subjects to have experienced pre- or perinatal adverse events (Waddington *et al.*, 1999), such as obstetric complications and particularly low birth weight and other measures of fetal growth restriction (Cannon *et al.*, 2002; Geddes & Lawrie, 1995; Hultman *et al.*, 1997a; Kunugi *et al.*, 2003; McNeil, 1995). The excess risk for obstetric complications in individuals who later develop schizophrenia has been interpreted as the result of early cerebral insult. Thus, it is important to elucidate whether associations between complications such as low birth weight and/or fetal growth restriction and offspring's risk for schizophrenia is mediated by genetic, intrauterine environment, and/or shared environmental effects later in life.

AIMS OF THE THESIS

The overall aims were to investigate the importance of genetic and environmental factors in the development of adverse pregnancy outcomes and to investigate the role of schizophrenia as a risk factor for and a consequence of adverse pregnancy outcomes.

The specific aims were:

- To determine the relative importance of genetic and environmental effects in the etiology of pre-eclampsia and gestational hypertension, and to investigate whether pre-eclampsia and gestational hypertension share genetic etiology.

- To examine risks of adverse pregnancy outcomes among mothers and fathers with schizophrenia.

- To investigate if the increased risk for adverse pregnancy outcomes among women with schizophrenia are due to maternal, paternal, genetic and/or environmental factors.

- To investigate if the association between fetal growth restriction and subsequent development of schizophrenia is mediated by familial factors.

MATERIAL AND METHODS

SETTING

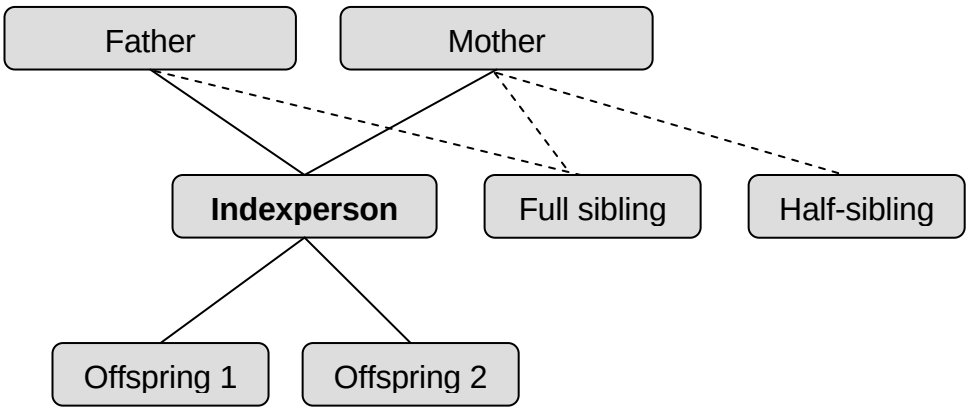
The studies in this thesis were all based on data from Swedish population-based registers. The long tradition of good population statistics, the structure of the Swedish health care system, and the nationwide health care registers provide an excellent basis for epidemiological research. Furthermore, the systematic use of the national registration number is a prerequisite for studies like these. The national registration number system was introduced in Sweden in 1947 as a unique ten-digit personal identifier (Lunde *et al.*, 1980). It was assigned to all residents alive in January 1, 1947 and has been assigned to all residents born or immigrated thereafter. The national registration number is used to link information from different sources.

DATA SOURCES

The Multi-Generation Register

The Multi-Generation Register was created by Statistics Sweden in the early 1990s by linkage of several different data sources, providing information on all first-degree relatives for residents born in Sweden 1932 or later (Statistics Sweden, 2001b). To be included in the register, index persons had to be alive in 1960 or born thereafter. Nine million index persons and their parents are included in the register and adoptions and other non-biological relations are flagged.

Figure 2. Example of an indexperson and first degree relatives.



The Medical Birth Register

The Swedish Medical Birth Register, held by the National Board of Health and Welfare, contains information from standardized antenatal, obstetric, and neonatal records from 1973 onwards (Centre for Epidemiology, 2003). The register includes almost all births in Sweden and only 1-2 per cent of the records are missing for most years. The information is collected prospectively. Maternal characteristics in the register include maternal age, information about previous pregnancies, smoking habits, and family situation early in pregnancy. Complications during pregnancy and delivery are coded according to the Swedish version of the International Classification of Diseases (ICD), 8th revision from 1973 through 1986, 9th revision from 1987 through 1996, and 10th revision from 1997 onwards (World Health Organization, 1967; World Health Organization, 1977; World Health Organization, 1992). Information about the infant includes vital status, birth weight, head circumference, birth length, gestational age, sex, Apgar score, and infant diagnoses.

The Hospital Discharge Register

The Hospital Discharge Register, held by the National Board of Health and Welfare, is based on individual information on inpatient hospital care, and the coverage is nation-wide for psychiatric care since 1973 (Centre for Epidemiology, 2005). For each occasion, the register includes date of admission, date of discharge, and main and up to eight contributory discharge diagnoses, coded according to the International Classification of Diseases, 8th through 10th revision.

The Cause of Death Register

The Cause of Death Register is kept by the National Board of Health and Welfare and has been computerized since 1952. The cause of death is generally determined from the medical death certificates completed by the attending physician or coroner. The register includes date of death, main and contributory cause of death coded according to International Classification of Diseases (8th through 10th revision). The completeness is estimated to exceed 99 per cent (Centre for Epidemiology, 2004).

The Education Register

Statistics Sweden established the Education Register in 1985 and the register includes information on the highest level of formal education for all individuals living in

Sweden between the ages of 16 and 74. The register is updated annually (Statistics Sweden, 2001a).

The Swedish Twin Register

The population-based Swedish Twin Register, held by Karolinska Institutet, includes information about twins born in Sweden since 1886 (Lichtenstein *et al.*, 2002). In 1972, the cohort of same-sexed twin pairs born 1926 to 1958 was approached and both members of 14,000 twin pairs completed a questionnaire. The study presented in this thesis included same-sexed twin-pairs born since 1926 and alive in the year 2000 with information on birth characteristics from medical records.

STUDY DESIGN

Table 1 provides an overview of the design of the studies included in this thesis. It describes the subjects, data sources, key comparisons, outcome measures, risk factors, and covariates included.

Table 1. Overview of the papers.

Paper	I	II	III	IV
Subjects	Single births from 1987 through 1997 (n=1,188,207)	Single births from 1983 through 1997 (n=1,555,975)	Single births from 1983 through 2002 (n=1,890,550)	Same-sexed twin pairs born 1926 onwards and alive in 2000 (n=11,360 twins)
Data sources	Medical Birth Register, Multi-Generation Register	Medical Birth Register, Hospital Discharge Register, Education Register	Medical Birth Register, Hospital Discharge Register, Education Register, Cause of Death Register, Multi-Generation Register	Twin Register, Hospital Discharge Register, Cause of Death Register, Medical records
Key comparisons	Full and half-sister pairs, mother-daughter pairs	Mothers	Parents and parental full and half-siblings	Unrelated and within-pair comparisons
Outcome measures	Pre-eclampsia, gestational hypertension, pregnancy-induced hypertension	Stillbirth, infant death, preterm delivery, low birth weight, SGA	Low birth weight, preterm delivery, SGA, infant death	Schizophrenia
Risk factors		Schizophrenia	Schizophrenia	Low birth weight, small head circumference, preterm delivery, congenital debility
Covariates		Maternal age, parity, cohabitation status, maternal smoking, pregnancy-induced hypertensive disease, maternal education	Maternal age, parity, cohabitation status, maternal smoking, parental education, schizophrenia in spouse	Congenital debility

Paper I

In order to determine the importance of genetic effects in the etiology of pre-eclampsia and gestational hypertension and to investigate whether pre-eclampsia and gestational hypertension share genetic etiology, we linked the Multi-Generation Register and the Medical Birth Register. All single births during the study period 1987 through 1997 were included and information from the Medical Birth Register was used to define whether the women had pre-eclampsia, hypertension during pregnancy, or was normotensive. If a woman had at least one pregnancy with pre-eclampsia she was classified into that category. The same held true for gestational hypertension. Sisters and mothers to a woman with pre-eclampsia or gestational hypertension were identified and pairs of healthy, disease discordant, and disease concordant pairs were created and used in the analyses (Table 1). A sibship that contained one affected and one unaffected woman was counted as one discordant pair, a sibship with two affected sisters was regarded as a concordant pair. A sibship with two affected and one unaffected woman was counted as one concordant and two discordant pairs. The sample consisted of 312,310 full sister pairs, 26,748 maternal half-sister pairs and 32,757 paternal half-sister pairs and 51,684 mother-daughter pairs.

Paper II

In this cohort study we investigated the risk for stillbirth, infant death, preterm delivery, low birth weight, and SGA births for women with schizophrenia through a record linkage between three population-based registers, the Medical Birth Register, the Hospital Discharge Register, and the Education Register (Table 1). Women with schizophrenia were identified through the Hospital Discharge Register from 1977 through 1997. We included 1,558,071 single births between 1983 and 1997. Women with schizophrenia were analysed in three ways; the first group consisted of all women with schizophrenia (regardless of whether they gave birth before or after they were diagnosed with schizophrenia, 2,096 births by 1,438 women with schizophrenia); the second group consisted of women who gave birth after they were diagnosed with schizophrenia (935 births by 696 women); and the third group consisted of women admitted to hospital for schizophrenia less than nine months before delivery (201 deliveries by 188 mothers). The covariates included in the study are listed in Table 1.

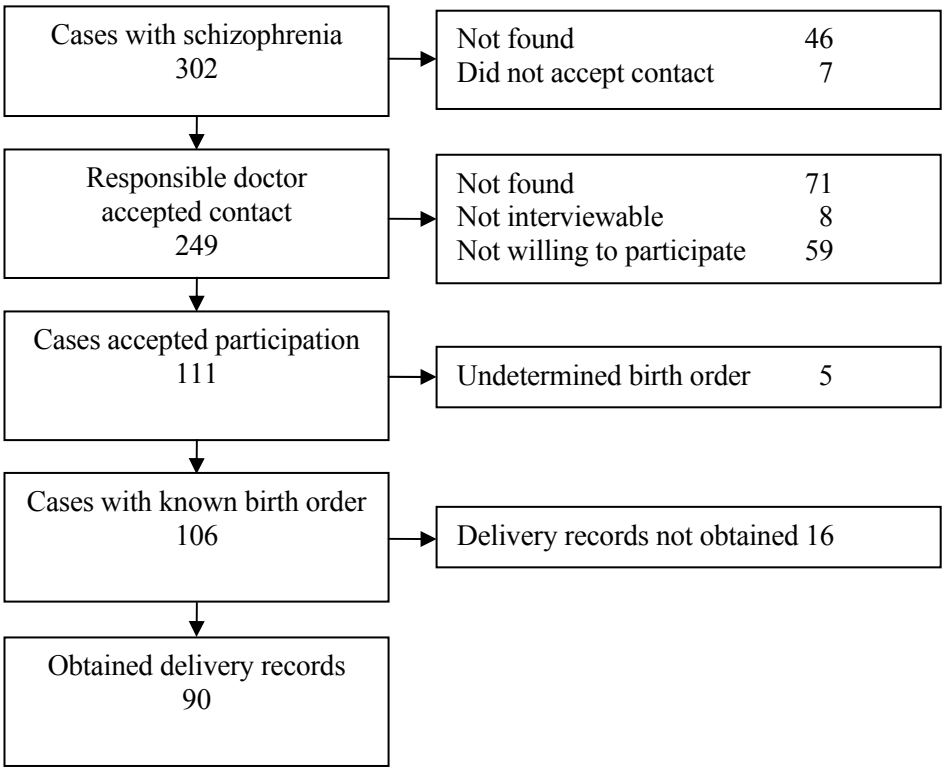
Paper III

In this study we investigated the mechanisms underlying the risk for parents with schizophrenia to have adverse pregnancy outcomes (low birth weight, preterm delivery, SGA, and infant death). We linked the Multi-Generation Register, the Hospital Discharge Register, the Medical Birth Register, the Education Register, and the Cause of Death Register (Table 1). The study population was defined by women giving birth to singletons from 1983 through 2002, in all 1,890,550 births. The father and parental siblings were identified through the Multi-Generation Register and all included individuals were linked to the Hospital Discharge Register to identify individuals with schizophrenia. Risks for adverse pregnancy outcomes related to maternal and paternal schizophrenia were estimated. By using information on schizophrenia in parental full and half-siblings, it was also possible to assess whether the risk could be explained by genetic factors (covariates included in the study are given in Table 1).

Paper IV

The fourth study was designed to investigate if infants born with low birth weight and/or fetal growth restriction had an increased risk for schizophrenia later in life. The population-based Swedish Twin Register was linked to the Hospital Discharge Register and the Cause of Death Register (Table 1). Same-sexed twin pairs, born after 1926 and alive in the year 2000 were eligible. We identified 302 cases with schizophrenia and verified the diagnosis by contacting the responsible doctors for every patient and asked them for permission to contact the twins with schizophrenia (Figure 3). Two hundred and forty nine doctors accepted contact with cases (46 responsible doctors were not found and 7 responsible doctors did not accept contact with cases) and 111 cases accepted (71 cases were not found, 8 cases were not interview able and 59 cases did not want to participate). Among those, we obtained information on birth order in 106 cases. We obtained the delivery records for 90 (85% out of 106) of the twins diagnosed with schizophrenia.

Figure 3. Sampling scheme for inclusion of twins with schizophrenia in paper IV.



The analysis of the association between birth characteristics and schizophrenia were divided into two parts; the first part of the analysis was a cohort-analysis including 11,360 same-sexed twin pairs born between 1926 and 1958 with information from birth records. Of these, 88 had a diagnosis of schizophrenia. The second part of the analysis was a nested case-control study of twin-pairs discordant for disease, and 82 discordant twin-pairs born between 1927 and 1974 were included.

OUTCOME MEASURES

Paper I

The prenatal care in Sweden is free of charge and all women have access to the same antenatal and obstetric care. The pregnant population is relatively homogeneous and maternal parity and age is not associated with number of visits to the prenatal care (Aberg & Lindmark, 1992). Registration to prenatal care generally occurs at 8 to 12 gestational weeks. Prenatal routines includes visits every fourth week up to 24 gestational weeks, then every second week up to 36 weeks and weekly thereafter. At each visit, blood pressure is measured and urine is checked for protein using a dipstick. Ninety per cent of the pregnant women visit antenatal care at least nine times (Aberg & Lindmark, 1992). Diagnoses during pregnancy are noted at the time of discharge from

the hospital, using a guide sheet where definitions of diagnosis are written in clear text besides the code from the International Classification of Diseases (ICD).

Pre-eclampsia

Pre-eclampsia was defined as gestational hypertension combined with proteinuria (two urinary protein dip sticks of at least 1+ or 300 mg of protein or more in a 24-hour urine collection). The diagnosis was coded according to the ICD 9th and 10th revision; ICD-9 codes 642E and 642F; and ICD-10 code O14.

Gestational hypertension

Gestational hypertension was defined as blood pressure increase during pregnancy to at least 140/90 mm Hg measured on at least two occasions occurring after 20 weeks of gestation. The diagnosis was coded according to the ICD 9th and 10th revision; ICD-9 codes 642D and 642X and ICD-10 codes O13 and O16.

Pregnancy-induced hypertension

Pregnancy-induced hypertension was defined as either gestational hypertension or pre-eclampsia and analysed as an ordinal scale with three values; (i) unaffected individuals, (ii) individuals with gestational hypertension, and (iii) individuals with pre-eclampsia.

The accuracy of the diagnosis in the Medical Birth Register for pre-eclampsia and gestational hypertension has previously been evaluated (Salonen Ros *et al.*, 2000). Among 115 pregnancies, coded as gestational hypertension according to the ICD-classification in the Medical Birth Register in 1987 and 1993, 97 women had the diagnosis according to the notes in the individual records, i.e., positive predicitive value 84 per cent. Among 148 pregnancies recorded as pre-eclampsia according to the ICD-classification, 137 women had pre-eclampsia, i.e., positive predictive value 93 per cent.

Paper II and III

Low birth weight

Low birth weight was defined as birth weight below 2,500 grams. Information on birth weight in the Medical Birth Register is lacking for 0.3 per cent of all infants. Birth weights between 300 and 7,000 grams were allowed. Weights between 300 grams and 1,499 grams were examined and compared to the infant's birth length, head

circumference, and gestational age. If this information was contradictory, the weight information was set to missing (Centre for Epidemiology, 2003).

Preterm delivery

Preterm delivery was defined as delivery before 37 weeks of gestation. Information on gestational age is primarily based on routine ultrasound examinations starting in the late 1980s. If this information is missing, the last menstrual period is used to calculate pregnancy duration. Before the ultrasound became widely used, the gestational age was estimated from the mother's information about the first day of the last menstrual period. Adequate data can be obtained with the above hierarchical system used for estimates of gestational duration (Centre for Epidemiology, 2003).

Small-for-gestational-age

Small-for-gestational age was defined as birth weights more than 2 SDs below the mean birth weight for gestational age and sex according to the Swedish reference curve of estimated fetal growth (Marsal *et al.*, 1996).

Infant death and stillbirth

A record linkage between the Medical Birth Register and the Cause of Death Register was made to retrieve information on infant death, that is, live born infants dying within the first year of life. Information on stillbirths was retrieved from the Medical Birth Register and defined as fetal death occurring at the 28^{th} week of gestation or later (only used in paper II). The number of stillbirths and infant deaths reported to the Medical Birth Register is, on a yearly basis, compared to the number of stillbirths and infant deaths registered at Statistics Sweden. From these comparisons, birth records that are not reported to the Medical Birth Register are retrieved from the hospitals and the register is updated. Therefore, the information on stillbirth and infant death in the Medical Birth Register is good.

Paper IV

Schizophrenia

Subjects with schizophrenia were defined as individuals identified in the Hospital Discharge Register with at least one hospital admission for schizophrenia and classified according to the International Classification of Diseases (World Health Organization, 1967; World Health Organization, 1977; World Health Organization, 1992). In paper

IV, ICD-8 and ICD-9 code 295 was used to identify schizophrenia patients. The discharge diagnosis of schizophrenia has been validated and few false positive cases have been reported (Ekholm *et al.*, 2005). A relatively high agreement between research DSM-IV diagnoses and register-based diagnoses has been observed for schizophrenia and schizophrenic psychosis (75 percent and 94 percent, respectively) and this relatively high magnitude of agreement has also been shown in previous studies of adult (Kristjansson *et al.*, 1987) and young (Dalman *et al.*, 2002) schizophrenic patients in Sweden.

RISK FACTORS

Paper II and III

Schizophrenia

Similarly as in paper IV, subjects with schizophrenia had at least one hospital admission for schizophrenia registered in the Hospital Discharge Register. The diagnoses were classified according to the *International Classification of Diseases*. We used the ICD-8 and ICD-9 code 295 and ICD-10 codes F20, F21, F23.1, F23.2, and F25 to identify individuals with schizophrenia.

Paper IV

Low birth weight

Twins are on average 800-900 grams lighter than singletons at birth and are delivered earlier (Leon, 2001). We divided the twin cohort in three categories according to the birth weight distribution in the cohort; ≤ 1999, 2000-2299 and ≥ 2300 grams, representing 10%, 15% and 75% of the cohort. As measure of fetal growth restriction, the birth weight ratio was created as the ratio of the observed to the expected birth weight for gestational age and sex. Birth weight ratio was categorized including 10%, 15%, and 75% of the cohort. Birth weights below 1500 grams were evaluated and compared to the infant's birth length, head circumference and gestational age. If this information was contradictory, the weight information was set to missing.

Head circumference

We also used small head circumference as an indicator of fetal growth. Small head circumference was defined as belonging to the lowest quartile, i.e. less than 31.5 cm. Head circumference ratio was created in the same manner as birth weight ratio, and low head circumference ratio included the lowest quartile.

Preterm delivery

Preterm delivery was based on the last menstrual period, and was defined to include the lowest quartile of the twin-cohort, i.e., ≤ 37 gestational weeks.

Congenital debility

Signs of asphyxia at birth, weakness after delivery and the need for a child to remain under care after birth were noted with the diagnosis '*congenital debility*' (coded according to the *Manual of International Statistical Classification of Diseases, Injuries, and Causes of Death,* 6th and 7th revision code no 772.0 and 773.0). We have used this diagnosis as a proxy for Apgar score, which is the current standardized assessment of asphyctic signs at one, five and ten minutes after delivery.

COVARIATES

Paper II and III

The information on covariates in paper II and III was collected from the Medical Birth Register, except for 'level of education' which was retrieved from the Education Register.

Maternal age

Maternal age was defined as completed years at the time of delivery, categorized in ≤ 24, 25-29, 30-34, and ≥ 35 years.

Parity

Parity was defined as the number of children preceding the pregnancy under study and categorized as 1, 2-3 and ≥ 4 children.

Maternal smoking

The variable was categorized as non-daily smoking, 1-9 cigarettes per day, and more than 10 cigarettes per day. Information on smoking is collected at the first visit to antenatal care, which in 95 per cent of the pregnancies occurs before the 15th week of gestation. The information on maternal smoking is available in the Medical Birth Register since 1983, the validity is good and information is lacking in 4 to 9 per cent of all pregnancies (Centre for Epidemiology, 2003).

Cohabitation status

The information on family situation is collected at the first visit to antenatal care and was categorized into living with the infant's father or having another family situation. The information on cohabitation status has a good quality with missing data in about five per cent of all pregnancies (Centre for Epidemiology, 2003).

Mother's country of birth

The information about mother's country of birth was divided into three categories; the first category consisted of the Nordic countries (Sweden, Norway, Denmark, Finland and Iceland), the second category included Western Europe, the United States, Canada, Australia and New Zealand, and the last category included women born in other countries (only in Paper II).

Pregnancy-induced hypertensive disease

The information on pregancy-induced hypertensive diseases was classified by the physician at the time of discharge from hospital. It was coded according to the *International Classification of Diseases*; ICD-8 code 637, ICD-9 code 642 and ICD-10 codes O10-O15 (only in Paper II).

Level of education

The parent's highest level of formal education was defined as number of completed years at school as recorded in the Education Register. Information about education was grouped into ≤ 9, 10-11, 13-14, and ≥ 15 years. Paper II included the mother's highest level of formal education until Dec. 31, 1997, and paper III included both parents' highest level of formal education until Dec. 31, 2001.

STATISTICAL METHODS

Paper I

Classical twin analysis attemps to estimate the relative contribution of genetic and environmental influences for a trait or disease. This is done by comparing monozygotic twins (MZ), who are genetically identical, with dizygotic twins (DZ), who share on average 50 per cent of their segregating genes. That is, if MZ twins are more similar than DZ twins, this difference can be attributable to genetic factors since similarity with regard to environmental components is assumed to be the same for both MZ and DZ pairs (equal environment assumption). In this study we extended the twin design to families. Pairs of full sisters, maternal and paternal half-sisters and mother-daughter pairs were created. The analyses were based on the assumptions that the correlation between full sisters and mother-daughter pairs depends on common genes (average 50 per cent) and a common family environment, the correlation between half-sisters with the same mother depends on common genes (average 25 per cent) and a common family environment, and the correlation between half-sisters with the same father depends on common genes (average 25 per cent) only, since most children continue to live with their mother.

If genetic effects are present in the etiology of pre-eclampsia and gestational hypertension we expect the risks among first-degree relatives to be higher than second degree relatives. To investigate this, the risk of pre-eclampsia for women whose sister had been diagnosed with pre-eclampsia was compared with women whose sister had not been diagnosed with pre-eclampsia. These risks were calculated separately for the different types of sister pairs. For mother-daughter pairs, we estimated the risk of pre-eclampsia among daughters to mothers who had been diagnosed with pre-eclampsia, compared with the risk of daughters whose mother had not been diagnosed with pre-eclampsia (the same procedure was applied to gestational hypertension). Similarly, to test for co-morbidity, we calculated the risk of pre-eclampsia for women whose sister had been diagnosed with gestational hypertension, compared with women whose sister had not been diagnosed with gestational hypertension. The same principle was applied for all pairs of relatives. The risk was estimated as an odds ratio (OR) and confidence intervals (95%) were estimated according to Mantel-Haenszel's method (Kuritz *et al.*, 1988).

The majority of the complex diseases are assumed to be influenced by multiple genes and environmental factors and for these conditions a liability-threshold model is often assumed (Neale & Cardon, 1992). An underlying continuous variable, the liability, is assumed to have a normal distribution in the general population. A threshold value is calculated from the prevalence of the disease and the disease is thought to be present when an individual's liability exceeds the threshold value. The similarity between relatives was calculated as a tetrachoric (pre-eclampsia and gestational hypertension) or polychoric correlation (pregnancy-induced hypertension). These measures are sometimes referred to as correlation in liability (Neale & Cardon, 1992). The importance of genetic effects was indicated by higher correlation among first-degree compared to second-degree relatives.

Structural model fitting techniques were used to estimate the relative importance of genetic and environmental components of variance in liability to disease. To examine the nature of the familial aggregation we used the equation P = A + C + E, where P is the liability to disease, A is the additive genetic factor (heritability; the proportion of phenotypic variance explained by genetic variance (Plomin *et al.*, 2001)), C is the shared family environment (assuming it is equal in mother-daughter and sister relations) and E is the non-shared environment. Shared family environment can in turn be divided into sister-specific (Cs) and a common family component (Cc). The equation then becomes; P = A + Cc + Cs + E. The equations for the expected correlations are shown in Table 2. By using data from all relatives simultaneously and comparing the covariances by degree of relative, the different components of variance were estimated using the Mx program (Neale *et al.*, 1999).

Table 2. The equations for the expected correlations, by degree of relatedness.

Pairs of relatives	**Correlation**	**A+**	**Cs+**	**Cc+**	**E**
MZ	ρ_{MZ}=	1	1	1	1
DZ	ρ_{DZ}=	.5	1	1	1
Full sisters	ρ_{FS}=	.5	1	1	1
Maternal half-sisters	ρ_{MHS}=	.25	1	1	1
Paternal half-sisters	ρ_{PHS}=	.25	0	0	1
Mother-daughter	ρ_{MD}=	.5	0	1	1

Paper II and III

Multivariate logistic regression analyses were used to estimate the risk for adverse pregnancy outcomes in mothers (paper II, III) with schizophrenia. Also, we investigated if the risk for adverse pregnancy outcomes was increased if the father or parental sibling had schizophrenia (paper III).

Since some women had more than one pregnancy we needed to control for the dependence between these pregnancies using Generalized Estimation Equation models (GEE), which provides a correlation structure within an observation (Diggle *et al.*, 1994). In paper II, due to computational reasons, we were only able to analyze a subsample of the data with GEE models (n=43,510 births). These analyses left all relative risks and confidence intervals essentially unchanged. In paper III, we used the full sample to calculate the descriptive statistics. However, due to computational reasons, all individuals with schizophrenia and random samples of 300,000 births were analyzed in the GEE models.

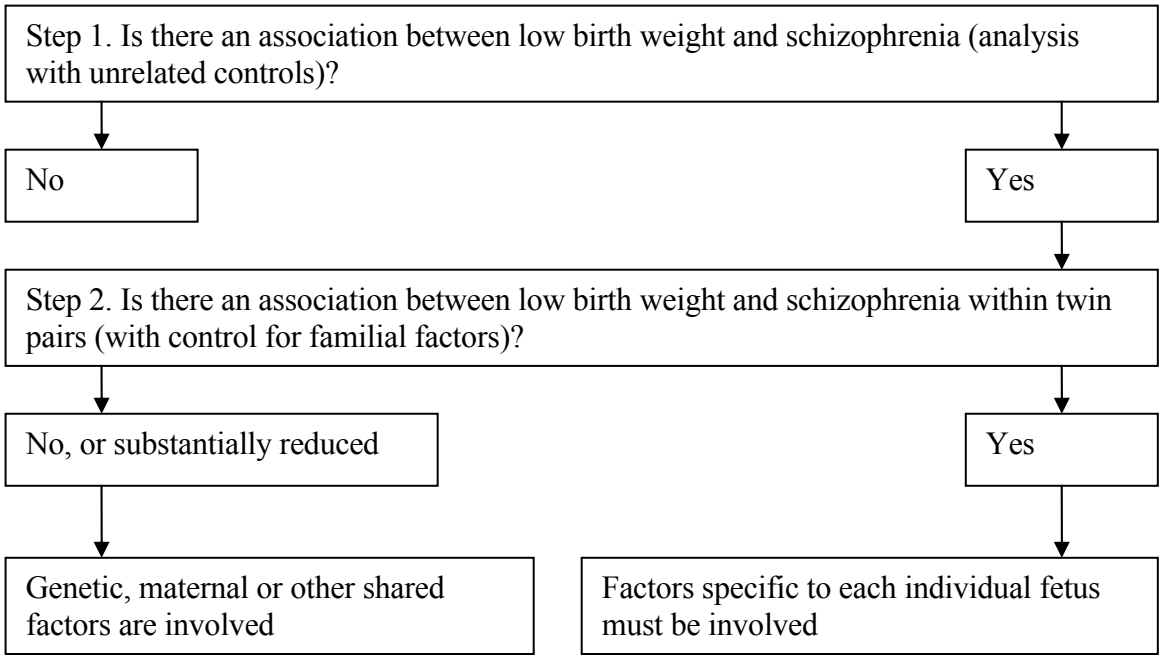
In paper III we wanted to investigate whether the risk for adverse pregnancy outcomes could be due to maternal and/or genetic factors, interaction effects were calculated. A *maternal factor* was indicated if the risk was significantly higher for mothers with schizophrenia compared to fathers with schizophrenia. A *genetic factor* was identified if both parents with schizophrenia had an increased risk for adverse pregnancy outcomes and if the risk was significantly higher for parental full siblings compared to parental half-siblings.

Paper IV

In paper IV we wanted to elucidate whether genetic and/or environmental factors were important for the increased risk for schizophrenia among infants with adverse pregnancy outcomes. The analyses in this study were done in two steps (Figure 4). In the first step we used controls that were unrelated to the cases. These analyses were performed using logistic regression. In order to take the dependence within twin pairs into account we analyzed the data using GEE.

In the second step, within-pair analyses were performed with conditional logistic regression. The co-twin control design allows us to control for confounding familial factors and by comparing disease discordant twins we could determine whether individual specific or shared factors were operating.

Figure 4. Cohort and within-pair analyses.



RESULTS

GENETIC AND ENVIRONMENTAL INFLUENCES ON PRE-ECLAMPSIA AND GESTATIONAL HYPERTENSION (PAPER I)

The Medical Birth Register and the Multi-Generation Register were used to estimate the relative importance of genetic and environmental effects in pre-eclampsia and gestational hypertension and to investigate if the two diseases share etiology.

Of the total number of 1,188,207 births during the study period 1987 through 1997, 2.8 per cent had pre-eclampsia and 1.1 per cent had gestational hypertension. Compared with a woman whose full sister did not have pre-eclampsia, a woman whose full sister had pre-eclampsia had a more than threefold increased risk of pre-eclampsia (Table 3). A similar increase in risk was observed for daughters to a mother with a diagnosis. The risk for half-sisters was lower and not statistically significant. The same pattern of associations was observed for gestational hypertension. Relatives with higher genetic similarity had higher risk for gestational hypertension as compared to relatives with lower genetic similarity.

Table 3. Odds ratio (OR) and 95% conficence interval (CI) for pre-eclampsia and gestational hypertension among sister-pairs and mother-daughter pairs.

	Pre-eclampsia		**Gestational hypertension**	
	OR	CI	OR	CI
Full sister	3.3	3.0-3.6	3.8	3.1-4.7
Half-sister (mother)	1.4	0.9-2.2	2.7	1.2-6.0
Half-sister (father)	1.0	0.6-1.6	1.3	0.5-3.5
Mother-daughter [a]	2.6	1.6-4.3	3.2	1.2-8.8

[a]The mother has the diagnosis, but not the daughter.

To study if there was a common etiology of gestational hypertension and pre-eclampsia, we analysed the risk of developing pre-eclampsia for a woman whose relative had gestational hypertension and *vice versa* (Table 4). Among women whose full sisters had pre-eclampsia there was a more than doubled increase in risk to develop gestational hypertension. Daughters had an increased risk to be diagnosed with pre-eclampsia given that the mother had gestational hypertension. No increased risk of co-morbidity was observed for maternal or paternal half-sisters.

Table 4. Odds ratio (OR) and 95% confidence interval (CI) for the co-morbidity of pre-eclampsia and gestational hypertension.

	OR	95%, CI
Full sister	2.5	2.2-2.8
Half-sister (mother)	1.0	0.6-1.8
Half-sister (father)	0.8	0.5-1.4
Mother-daughter[a]	2.7	1.5-4.7

[a]The risk for the daughter to be diagnosed with pre-eclampisia given that the mother had gestational hypertension.

In Table 5 the relative importance of genetic and environmental components is shown. For pre-eclampsia, the genetic effects accounted for 31 per cent of the variation in liability for pre-eclampsia and the non-shared environment accounted for 63 per cent. For gestational hypertension, genetic effects accounted for 20 per cent although not statistically significant. Non-shared environment accounted for 69 per cent. The estimates of shared environment were low and non-significant for both pre-eclampsia and gestational hypertension.

The co-morbidity analyses indicated a shared etiology between pre-eclampsia and gestational hypertension. We therefore performed quantitative genetic analyses of pregnancy-induced hypertensive diseases, with pre-eclampsia and gestational hypertension as different degrees of disease severity (Table 5). In these analyses the genetic effect accounted for 28 per cent of the variation in liability, the shared sister specific effect accounted for 7 per cent, and the non-shared environmental factor accounted for 64 per cent.

Table 5. Estimates of genetic and environmental effects for pre-eclampsia, gestational hypertension, and pregnancy-induced hypertension (PIH) from structural equation model fitting.

	Parameter estimates (95% CI) [a]			
	h^2	c_c^2	c_s^2	e^2
Pre-eclampsia	0.31 (0.09-0.45)	0.00 (0.00-0.00)	0.06 (0.00-0.13)	0.63 (0.55-0.74)
Gestational hypertension	0.20 (0.00-0.46)	0.06 (0.00-0.23)	0.05 (0.00-0.19)	0.69 (0.54-0.83)
PIH	0.28 (0.17-0.34)	0.00 (0.00-0.00)	0.07 (0.07-0.07)	0.64 (0.60-0.67)

Note: h^2= Genetic factor, c_c^2 = shared environment by the entire family (mother and daughter pairs as well as sister pairs), c_s^2 = sister environment, e^2 = non-shared environment.
[a]The fit of the models was tested with RMSEA and was ≤ 0.005 for all models, a good fit is indicated by a value of less than 0.05.

SCHIZOPHRENIA AND ADVERSE PREGNANCY OUTCOMES – MATERNAL, PATERNAL, AND GENETIC INFLUENCES (PAPER II AND III)

In paper II, the Medical Birth Register, the Education Register, and the Hospital Discharge Register were linked in order to investigate the risk for adverse pregnancy outcomes among women with schizophrenia. The cohort consisted of all 1,555,975 single births between 1983 through 1997. Of these, 2,096 infants had a mother with schizophrenia.

Compared to women without schizophrenia, women with the disease more often had high maternal age, were more likely to deliver their first infant, to have lower education, were more often smokers during pregnancy, and were less likely to cohabit with the infant's father.

Offspring to women with schizophrenia had an increased risk for stillbirth, infant death, low birth weight, and for being small-for-gestational age. Moreover, women with schizophrenia had an increased risk for preterm delivery (Table 6). Next, we controlled for covariates known to be more prevalent among women with schizophrenia compared to non-schizophrenic women. The risks decreased, but remained significantly increased for all outcomes, except for SGA.

Table 6. Crude and adjusted odds ratios for adverse pregnancy outcomes among women with schizophrenia compared to non-schizophrenic women.

	Risk for adverse pregnancy outcomes			
	Crude		Adjusted[a]	
	OR	CI	OR	CI
Stillbirth	2.1	1.3-3.5	1.6	1.0-2.7
Infant death	2.5	1.7-3.7	2.0	1.4-3.0
Preterm delivery	1.7	1.4-2.0	1.4	1.2-1.7
Low birth weight	1.8	1.5-2.2	1.3	1.1-1.6
Small-for-gestational-age	1.6	1.3-2.0	1.1	0.9-1.4

[a]Adjusted for maternal age, parity, maternal education, cohabiting with the infant´s father, mother´s country of birth, maternal smoking, and pregnancy-induced hypertensive disease.

In an attempt to investigate the most severe cases with schizophrenia, we restricted the analysis to women admitted to hospital for schizophrenia during pregnancy. Compared to women without schizophrenia, women admitted to hospital for schizophrenia during pregnancy had, after adjustment for covariates, a more than doubled increased risk of preterm delivery and low birth weight. A doubled, however not statistically significant, risk for stillbirth and infant death could also be observed.

In order to further elucidate the underlying mechanism of the increased risk for adverse pregnancy outcome among women with schizophrenia we undertook a record linkage between the Medical Birth Register, the Hospital Discharge Register, the Cause of Death Register, the Multi-Generation Register, and the Education Register (Paper III). All 1,890,550 single births between 1982 and 2002 were included, and the infant's parents and parental siblings were included in the study.

As in paper II, women with schizophrenia had an increased risk for adverse pregnancy outcomes (Table 7). After adjustments for covariates (e.g., schizophrenia in spouse, maternal smoking, and parental education) the risks decreased but remained significantly increased for preterm delivery and infant death. The risk for low birth weight, SGA, and infant death was increased if the father had schizophrenia. The risks decreased after adjustments, but remained significantly increased for SGA and infant death.

Table 7. Crude and adjusted odds ratios for adverse pregnancy outcomes among mothers and fathers with schizophrenia compared to non-schizophrenic parents.

	Risk for adverse pregnancy outcomes			
	Crude		Adjusted[a]	
Type of relative with schizophrenia	OR	CI	OR	CI
Low birth weight				
Mother	1.6	1.4-1.9	1.1	0.9-1.4
Father	1.3	1.1-1.6	1.1	0.9-1.3
Preterm delivery				
Mother	1.6	1.4-1.8	1.2	1.0-1.4
Father	1.1	1.0-1.4	1.0	0.8-1.2
Small-for-gestational-age				
Mother	1.4	1.2-1.7	0.9	0.7-1.2
Father	1.5	1.3-1.9	1.2	1.0-1.6
Infant death				
Mother	2.4	1.7-3.4	2.2	1.5-3.2
Father	1.9	1.2-2.8	1.8	1.1-2.8

[a]Adjusted for schizophrenia in spouse, maternal age, parity, maternal and paternal education, cohabitation status, maternal smoking.

We also studied causes of infant death since infants born to a parent with schizophrenia had an increased risk of death within the first year of life. Compared to death within the first year of life among offspring to parents without schizophrenia, no statistically

significant difference in cause of deaths (sudden infant death syndrome or congenital malformations) was observed for offspring to mothers and/or fathers with schizophrenia. However, infants with a parent with schizophrenia were more likely do die postneonatally (28-364 days after birth), compared to offspring to parents without schizophrenia. Among in all 57 infant deaths to a mother and/or father with schizophrenia, 31 infants (54.4 per cent) died postneonatally, while among 8,342 infant deaths in offspring to parents without schizophrenia, 3,197 (38.3 per cent) died postneonatally (p=0.01).

In order to illuminate whether a genetic factor contributes to the association between parental schizophrenia and adverse pregnancy outcomes, we compared the risk for adverse pregnancy outcomes in parental full and half-siblings with and without schizophrenia. Compared to full siblings (full sisters or full brothers) without schizophrenia, the risk for low birth weight was slightly increased if the full sibling had schizophrenia. The risk was not increased if the half-sibling (half-sisters or half-brothers) had schizophrenia. The risk for low birth weight was significantly higher if the parental full sibling had schizophrenia as compared to if the parental half-sibling had schizophrenia (p=0.01), indicating a genetic factor. No significant genetic factor was found for preterm delivery, SGA, or infant death.

FETAL GROWTH RESTRICTION AND RISK FOR SCHIZOPHRENIA (PAPER IV)

In paper IV, we investigated whether the association between fetal growth restriction and schizophrenia was due familial factors. In a cohort of 11,360 same-sexed twins born between 1926 and 1958, we found that twins with low birth weight and twins with small head circumference had an increased risk for schizophrenia (Table 8). The associations decreased, but remained elevated (not statistically significant), when the association between fetal growth restriction and schizophrenia was analysed in the cohort.

The increased risk for schizophrenia remained in the within twin pair comparison designed to take unmeasured familial factors into account. These analyses included 82 pairs discordant for schizophrenia (Table 8). Also, the increased risk for schizophrenia among infants with small head circumference remained in the within twin pair comparison (not statistically significant).

We also found an association between congenital debility and risk of schizophrenia. When we used external controls, children with debility had, in the crude analysis, an almost doubled risk of developing schizophrenia compared to children without debility (Table 8). After adjustment for gestational age the risk decreased, but was still elevated (not statistically significant). In the within pair analysis, debility was associated with a doubled, although not statistically significant, increased risk of schizophrenia.

Table 8. Crude and adjusted odds ratios for the association between birth weight and head circumference and risk for schizophrenia.

	Cohort analysis[a]		Cohort analysis with control for gestational age and sex[b]				Within-pair comparison[c]	
	Crude		Crude		Adjusted[d]		Crude	
	OR	CI	OR	CI	OR	CI	OR	CI
Birth weight								
Very low	1.7	0.9-3.1	1.4	0.8-2.7	1.3	0.7-2.6	1.5	0.6-3.7
Moderately low	1.8	1.1-3.1	1.1	0.6-2.0	1.0	0.6-1.8		
Normal	Ref		Ref		Ref		Ref	
Head circumference								
Small	1.6	1.0-2.5	1.4	0.9-2.2	1.3	0.8-2.1	1.7	0.6-4.6
Normal	Ref		Ref		Ref		Ref	
Congenital debility								
Yes	1.9	0.99-3.5			1.6[e]	0.8-3.0	2.0	0.2-22.1
No	Ref				Ref		Ref	

[a] Birth weight; very low ≤1999, moderately low 2000-2299, normal ≥ 2300.
Head circumference; small ≤ 31.5, normal ≥ 32.0.
[b] Birth weight; very low 10%, moderately low 15% and normal 75% of the cohort.
Head circumference; small 25%, normal 75% of the cohort.
[c] Birth weight; low ≤ 2299, normal ≥ 2300. Head circumference; small ≤ 31.5, normal ≥ 32.0.
[d] Adjusted for congenital debility.
[e] Adjusted for gestational age.

DISCUSSION

METHODOLOGICAL CONSIDERATIONS

Cohort studies are well suited for studies of rare exposures. The possibility of obtaining a high internal validity compared to other epidemiological studies, such as case-control studies, is good as the risk of selection bias is low. Also, the risk for selective recall of exposure information is low, since the information on exposures and outcomes in cohort studies can be obtained independently (e.g., through population-based registers). The Swedish Medical Birth Register is one of the population-based registers that provide the opportunity to perform retrospective cohort studies where exposure and disease information has been collected prospectively. Traditionally, the main objective against cohort studies is the efficiency. Cohort studies are often time-consuming and expensive, since only a minority of those who are at risk actually develops the disease. This argument does not apply to retrospective cohort studies using registry-based follow-up. A limitation of the retrospective register-based cohort studies is that we are restricted to information on exposure and potential confounders included in the register. All four studies in this thesis were based on the retrospective cohort design. Papers I through III were based on cohorts from the Medical Birth Register, and paper IV on a cohort from the Swedish Twin Register.

The following sections will briefly describe the two main sources of error in epidemiological studies: systematic errors, often referred to as bias, affecting the validity of the results, and random errors, affecting the precision of the results. Thereafter, some of the assumptions in twin methodology will be discussed.

Validity

The validity in an epidemiologic study may be defined as the degree of systematic error in a study that results in an incorrect estimate of the association between exposure and outcome. Internal validity is commonly classified in three categories: selection bias, information bias, and confounding. The external validity is referred to as the possibility to generalize the results to other populations than that under study.

Selection bias

Selection bias is a systematic error emanating from the procedure of selecting study subjects. Selection bias is present when the association between exposure and disease

differs for those who participate and those who do not participate in the study. In cohort studies, selection of study subjects is not a source of bias, but a bias may occur if there are systematic differences between participants and non-participants due to non-response or loss of follow up. This selection bias in cohort studies is sometimes viewed as a matter of external validity rather than internal validity. In paper I, II and III, the cohorts were based on the Medical Birth Register which includes between 97 and 99 per cent of all births yearly (Centre for Epidemiology, 2003). The possibility of a systematic error influencing the outcome caused by this relatively low number of loss to follow up is not likely. Paper IV was based on a cohort of twins from the Swedish Twin Register, which covers nearly all twins born in Sweden since 1926. Twins with schizophrenia (302 individuals) were identified through the Hospital Discharge Register and the diagnosis was verified by contacting the responsible doctor for every patient and asking for permission to contact the twins with schizophrenia. Two hundred and forty nine doctors accepted contact with cases and 111 cases accepted. Two sources of selection bias could be present in this ascertainment process. First, 46 responsible doctors were not found, and 7 did not accept contact with the patient. These patients could have differed from the patients included in the study with regard to severity of disease, either as too ill to be contacted or too well to be in contact with their doctors and thereby the doctors were unable to contact the patient. Second, twin pairs in which at least one twin in a pair had schizophrenia was approached in a telephone interview in order to disentangle the birth order and thereby ascribing the correct birth weight to the twin with schizophrenia. This procedure led to a loss to follow up of cases that did not want to participate. It is not clear whether this selection bias could have influenced the estimates, although it is clear that this loss to follow up affected the precision of the study.

Information bias

Systematic error can arise because the information collected about the study subjects is incorrect. These errors are often referred to as recall, detection, and/or misclassification bias. The bias due to *differential recall* was minimized in papers I through III since the cohorts were based on prospectively collected data from the Medical Birth Register and the diagnoses used were classified by the physician in the Hospital Discharge Register. The information about paternity was based on recordings of 'stated biological father' in the Multi-Generation Register (paper III). As self-reported information about paternity will lead to misclassification of paternity (Lucassen & Parker, 2001), our estimates

could have been biased. An overrepresentation of infants who did not have information about the identity of the father had a mother with schizophrenia, this is in line with the finding that women with schizophrenia are less likely to cohabit with the infants' father. This could have diluted the estimates in the analyses of fathers with schizophrenia.

Paper IV was based on the Swedish Twin Register. Information on exposure and outcome was assessed from medical records and the Hospital Discharge Register, thereby eliminating the risk for bias due to differential recall. To ascribe the correct birth weight to the twins in a pair, twins who later developed schizophrenia were approached in a telephone interview asking for the birth order of the twins. A higher risk for schizophrenia was obtained when we restricted the analyses to pairs whose birth order was confirmed in written documents (i.e., baptized and named at birth) compared to the analyses where all pairs were included, indicating that birth order collected through the interview may have included some misclassification, which may have led to an underestimation of the true risk.

The relatively low prevalence of gestational hypertension (paper I) may be an indication of *detection bias.* It is possible that gestational hypertension only has been reported when it was a more serious condition (higher blood pressure), or when it received more attention perhaps because of a known family tendency. This may have elevated the risk and exaggerated the familiality of the disease. The same may affect the results on the risk of disease development when comparing gestational hypertension and pre-eclampsia. However, blood pressure is measured on all women at every visit to antenatal care. It is therefore unlikely that women with a family history of gestational hypertension or pre-eclampsia would be diagnosed more often than women without a family history of the diseases.

Misclassification of exposure and outcome can be both differential and non-differential. Exposure misclassification is called non-differential if it is unrelated to disease classification, and differential if it is different for those with and without disease. Outcome misclassification is similarly differential if it is different for those exposed and unexposed to the variables under study. If the measurement error is systematic, the bias is referred to as differential misclassification and the error may be in any direction. A random error is non-differential and usually dilutes any observed risk estimate.

The information on pregnancy outcomes in paper I-III were based on the Medical Birth Register which covers in principle all deliveries in Sweden. The influence of a possible detection bias, which leads to misclassification of pre-eclampsia and gestational hypertension in paper I is described earlier in this section. The risk of misclassification of the adverse pregnancy outcomes in papers II and III is of course possible. However, the impact of the misclassification of these pregnancy outcomes is not likely to have influenced the result of the studies. In papers II, III, and IV information on schizophrenia is collected from the Hospital Discharge Register, which includes all in-patient care in Sweden. The diagnoses of schizophrenia in the Hospital Discharge Register are made by the treating psychiatrist, and the research group was not able to validate the diagnoses. However, the Swedish diagnostic practices of schizophrenic psychosis are considered to reflect diagnostic caution rather than overinclusivness, and few false positive cases are reported (Ekholm *et al.*, 2005). However, the number of hospitalizations for schizophrenia has decreased since the early 1990s. This is not due to a reduced number of cases with schizophrenia, but to a restructuring of the psychiatric care in Sweden. Patients with schizophrenia are to a greater extent treated in out-patient care than earlier. This has increased the probability that women with schizophrenia, not treated in the in-patient care have been misclassified as non-schizophrenic. In papers II and III, a misclassification, i.e., including women with schizophrenia in the unexposed group can not be ruled out, and risk estimates are likely to be underestimated. Similarly, a misclassification of schizophrenia as the outcome measure in paper IV can not be excluded.

Confounding

The definition of a confounding factor is that it influences the risk between exposure and outcome. A confounding factor is associated with the exposure and also an independent risk factor for the outcome. The confounder should not be an intermediate step in the causal pathway between exposure and outcome. There are several ways in which to control for the influence of a confounder: stratification of the data, matching for the confounder, and adjusting for the confounder in the multivariate analysis. In papers II and III we controlled for a number of potential confounders by including them in a multivariate analyses. However, some of the possible confounders included in the multivariate analyses may have been poorly defined. For instance, adjusting for educational and cohabitation status does not cover all aspects of socio-economic status. Although we adjusted for smoking in early

pregnancy, schizophrenic women may have continued to smoke more often during pregnancy than other women. In paper II, an increased risk for women with schizophrenia to have adverse pregnancy outcomes was found. However, we were not able to control for maternal medication, alcohol use during pregnancy and other possibly confounding factors, which could have influenced the association. In paper III, the potential confounding factors of maternal disadvantageous lifestyle during pregnancy were indirectly controlled for by analyzing fathers with schizophrenia. By showing an increased risk for adverse pregnancy outcomes among offspring to fathers with schizophrenia, we could show that not only maternal behaviour or medication could explain the association between schizophrenia and adverse pregnancy outcomes.

Generalisability

A high external validity or generalisability is achieved when the findings are applicable to people outside the study population, given a high internal validity. All studies included in this thesis are population-based, therefore the results should apply to all individuals in the catchment area fulfilling the inclusion criteria. Papers I-III were based on the Medical Birth Register, which covers practically all deliveries in Sweden and the results are therefore likely to apply to the whole population. However, a few threats to the generalisability should be mentioned. In paper I we were not able to include all women's first pregnancy due to limitations in the diagnostic criteria for the disease. The risk for gestational hypertension has previously been shown to be highest in the first pregnancy, therefore the results may not be generalisable to all women giving birth in Sweden. Furthermore, the low prevalence of gestational hypertension suggests underreporting, which also limits the generalisability of the results.

In paper IV we used a cohort of twins, and found that twins with low birth weight had an increased risk for schizophrenia later in life. This finding is in accordance with results in singletons. However, the generalisability of twin studies to the general population has been debated. Twins are on average 800-900 grams lighter than singletons at birth and are delivered 2-3 weeks earlier. Although they have a shorter gestation, their weight at birth is still smaller than singletons of similar gestational age (Leon, 2001). The intrauterine growth in twins is characterized by a normal development in the first two trimesters and a reduced growth during the last part of the pregnancy. The twins undergo a rapid catch-up growth in childhood, and studies of

anthropometric data have failed to find differences between twins and singletons in childhood or adolescence (Loos *et al.*, 2001; Pietilainen *et al.*, 1999). The generalisability has been particularly questioned in monozygotic twins. Monozygotic twins have lower birth weight than dizygotic twins, and are less concordant at birth than dizygotic twin pairs. Dizygotic twin pairs always have separate chorions and placentas that may or may not be fused. In contrast two-thirds of the monozygotic twins are monochorionic, i.e. they share placenta. Monochorionic monozygotic twins who share one placenta are more prone to develop twin-twin transfusion syndrome, which is an extreme form of unequal blood circulation, affecting approximately 10 per cent of monochorionic twins. However, a study including dizygotic, monozygotic dichorionic, and monozygotic monochorionic twin pairs failed to find evidence of any differences for most adult anthropometry indicating that chorion type does not bias the twin design (Loos *et al.*, 2001). Furthermore, there is no evidence that twins have increased overall mortality (Christensen *et al.*, 2001) compared to the general population. The generalisability of twin studies of schizophrenia has been analysed in two studies by Klaning et al., showing that dizygotic twins and being in a family with dizygotic twins are a risk factor for schizophrenia (Klaning, 1999; Klaning *et al.*, 2002), and suggests that the generalisabilty of twin studies of schizophrenia is questionable. However, other studies have not been able to find any differences in risk for schizophrenia between twins compared to singletons (Kendler *et al.*, 1996). Moreover, the incidence of schizophrenia did not differ across zygosity groups (Kendler *et al.*, 1996). Hence, it is still not fully elucidated whether the results from twin studies on schizophrenia are generalisable to the general population.

Precision

The error that remains if we could eliminate all systematic error is called random error. Random errors may give a deviation from the true value in an investigation, but would not give any deviation from the true value if the investigation was repeated an infinite number of times. A high level of precision or reliability in a study means a low influence of random error. Precision is improved with larger studies due to less variability in the estimates. The confidence intervals or statistical tests for significance provide information about the precision of the investigation, and judge the probability that a given result is due to chance. The sample sizes in paper I, II, and III were very large in this thesis. Therefore, the precision in these studies was relatively high, even though relatively rare exposures and outcomes were studied. The study described in

paper IV was of much smaller size, which may have been sufficient for the first part of the analyses, using the twin-cohort, but more limited in the within twin pair analyses of disease discordant twin pairs. The aim of the second part of the study was to control for genetic and environmental components by comparing genetically identical monozygotic twins with dizygotic twins (sharing on average 50 per cent of their segregating genes). Due to the limited precision we had to analyse the monozygotic and dizygotic twins together. This study highlights the difficulty in twin studies, even in quite large samples, to analyse discordant pairs in disorders with very high heritability estimates (Sharp *et al.*, 2003). Power calculations showed that to find a risk of 2, a sample of approximately 300 discordant twin pairs would be needed to have a power of 80 per cent at 5 per cent significance level. However, the results from the cohort and the within pair analyses were congruent, which indicates that genetic and shared environmental factors did not confound the association between fetal growth restriction and schizophrenia.

Twin methodological issues

Equal environment assumption

The equal environment assumption in the twin method assumes that environmentally caused similarity is roughly the same for both types of twins. If the assumption was violated because identical twins experience more similar environments than dizygotic twins, this violation would increase estimates of genetic influence. The equal environment assumption can be tested by studying twins who were misclassified with regard to zygosity by their parents or by themselves. The similarity in twins with mislabeled zygosity is compared to twins with the correct zygosity. Prenatally, monozygotic twins may experience greater environmental differences than dizygotic twins. The difference may be due to prenatal competition for those monozygotic twins who share the same chorion. The twin method will in this case overestimate the non-shared environment component. In paper I we have extended the twin method and included full and half-sister pairs as well as mother-daughter pairs. We assume that the shared environment component is the same for full sisters and maternal half-sisters, whereas paternal half-sisters do not share the same in-utero environment and are less likely to live in the same household. If this assumption was violated, this would have led to an underestimation of the shared environments for pre-eclampsia and gestational hypertension.

Assortative mating

Assortative mating refers to situations in which people select partners who are more similar for the phenotype than would be expected by chance. The twin method assumes non-random mating. Assortative mating tends to increase the similarity between dizygotic twins, thereby biasing the heritability estimates downward and the shared environment upward. The effect of assortative mating could be modeled if parental information is available. In paper I, the likelihood of assortative mating for pre-eclampsia or gestational hypertension is low.

FINDINGS AND INTERPRETATIONS

Genetic and environmental influences on pre-eclampsia and gestational hypertension

We found strong indications of a genetic liability for pre-eclampsia. Compared to women whose full sisters did not have pre-eclampsia, a woman whose full sister had pre-eclampsia had a threefold increased risk. The genetic effect of pre-eclampsia was further emphasised by the measures of similarity, which decreased with decreasing genetic similarity. The genetic effects accounted for 31% of the variation in liability to pre-eclampsia. A later study based on, in principle, the same database confirmed our results (Cnattingius *et al.*, 2004). This can be compared with the twin study of Salonen Ros et al., where the maternal genetic effect accounted for 54% of the variation (Salonen Ros *et al.*, 2000), and 22% from a British study of self-reported pre-eclampsia (Thornton & Macdonald, 1999), whereas two twin studies using medical records found no concordant twin pairs (Thornton & Macdonald, 1999; Treloar *et al.*, 2001). Thus, it is clear that pre-eclampsia is a heritable trait, where genetic effects account for a sizeable proportion of the liability.

The genetic component in the liability of gestational hypertension is less investigated and the studies are conflicting. Our study showed that compared to women whose full sisters did not have gestational hypertension, a woman whose full sister had gestational hypertension had an almost fourfold increased risk. Also, in agreement with the results of Salonen Ros et al. (2000), we found a non-significant genetic component of 20% of the variation in liability to gestational hypertension.

In the first paper, we investigated whether pre-eclampsia and gestational hypertension are different expressions of the same genetic or environmental liability. We found a

common familial component because women whose sister developed pre-eclampsia had significantly increased risks of developing gestational hypertension and *vice versa*. These results encouraged us to analyse pre-eclampsia and gestational hypertension as a trait with different degrees of disease severity. The quantitative genetic analyses showed that the estimated genetic effect on the liability of pregnancy-induced hypertension was 28%, whereas corresponding genetic estimate in the twin study amounted to 47% (Salonen Ros *et al.*, 2000).

Several differences have been described between gestational hypertension and pre-eclampsia, for example, level of pulse pressure in early pregnancy (Thadhani *et al.*, 2001) and hemodynamics (Bosio *et al.*, 1999). Pre-eclampsia is most often classified according to severity, indicated by the extent of proteinuria and blood pressure increase during pregnancy. However, it can also be classified by time of delivery, as preterm or term pre-eclampsia (Roberts & Cooper, 2001b; Roberts & Redman, 1993). Most cases of preterm pre-eclampsia are proposed to originate in poor placental perfusion. The maternal spiral arteries undergo extensive remodeling in the healthy pregnancy, and this remodeling is not complete in pre-eclamptic pregnancies. The second stage of pre-eclampsia is the transition to a maternal systemic disorder. Once pre-eclampsia is evident clinically, blood flow to virtually every organ in the pregnant woman, including the uterus, is reduced, resulting in the potential for multiorgan dysfunction. Several studies have argued that these pathological changes of pre-eclampsia are not those of hypertension (Lain & Roberts, 2002; Roberts & Redman, 1993). Compared to preterm pre-eclampsia, term pre-eclampsia may represent a mixture of conditions, ranging from moderate placental involvement to a hypertensive reaction to the burden of pregnancy (Roberts & Cooper, 2001b; Roberts & Redman, 1993).

On the other hand, previous reports suggest that a substantial fraction (46%) of women who develop gestational hypertension in early pregnancy also develop pre-eclampsia later during pregnancy, and around 10% progress to severe pre-eclampsia (Barton *et al.*, 2001). These studies indicate that, to some extent, the diagnosis of a woman is dependent on how soon after the occurrence of hypertension the delivery takes place. Moreover, compared with mild pre-eclampsia, severe gestational hypertension was associated with higher risks of preterm delivery and delivery of SGA infants. The development of mild hypertension or pre-eclampsia at or near term

is associated with minimal maternal and neonatal morbidity. In contrast, the onset of severe gestational hypertension and/or severe pre-eclampsia before 35 weeks' gestation is associated with significant maternal and perinatal complications (Buchbinder *et al.*, 2002; Hauth *et al.*, 2000). Moreover, data on similarities in risk factor patterns, including immunological (Wang *et al.*, 2002) and metabolic components (Innes *et al.*, 2001; Thadhani *et al.*, 1999), point out that it may be relevant to consider these diseases as etiologically related. Our results of a common liability for these two conditions give further evidence for a common genetic etiology.

One interpretation of the evidence for a shared etiology between the diseases could be that women with gestational hypertension could have developed pre-eclampsia if she had had her delivery later, especially in term pregnancies. We did not investigate this, but future studies of the shared etiology of pre-eclampsia and gestational hypertension should separate term and preterm pre-eclampsia in order to investigate if the shared etiology between the diseases is most prominent in the term pre-eclampsia cases. This will be important for the understanding of the causes of pregnancy-induced hypertensive diseases and as well as for designing studies searching for susceptibility genes.

Previous studies have indicated that paternal genes contribute to the risk of pre-eclampsia (Esplin *et al.*, 2001; Lie *et al.*, 1998). Cnattingius et al. (2004) found that more than 50% of the liability to pre-eclampsia was due to genetic factors and that the majority of these were attributable to maternal genetic effects. However, also fetal genetic effects and interaction effects between maternal and paternal genes were of importance (Cnattingius *et al.*, 2004). We have not been able to analyse the contribution of paternal genes. The effects of these genes are included in the model fitting analysis in the non-shared environment component, although we are not able to single out these effects from other non-shared environmental factors.

Schizophrenia as a risk factor for adverse pregnancy outcomes

In papers II and III we found, in line with previous studies, that women with schizophrenia are at increased risk for adverse pregnancy outcomes, such as stillbirth, infant death, preterm delivery, low birth weight, and SGA births (Bennedsen *et al.*, 1999; Jablensky *et al.*, 2005; Sacker *et al.*, 1996; Webb *et al.*, 2005). The increased risks could to some extent be explained by covariates (such as maternal smoking and single motherhood). However, for a number of outcomes the risks were still significantly increased even after adjustments. Women admitted to hospital for schizophrenia during pregnancy had the highest risks for adverse pregnancy outcomes, suggesting that acute psychosis during pregnancy is associated with higher risks of adverse pregnancy outcomes. Further, we showed increased risks for low birth weight, SGA, and infant death among offspring to fathers with schizophrenia, suggesting that factors mediated by the father also are of importance.

We have had the opportunity to control for factors known to influence risks for adverse pregnancy outcomes, which also are more prevalent among women with schizophrenia. It has been shown that women with schizophrenia often smoke, misuse other substances and are socio-economically disadvantaged (Bennedsen *et al.*, 1999), and these variables are well-known risk factors for adverse pregnancy outcomes (Cnattingius, 2004). Most of the increased risks for adverse pregnancy outcomes among parents with schizophrenia were accounted for by the covariates. Nevertheless, the risk for infant death was still increased for both mothers and fathers with schizophrenia, even after adjustment for covariates. Also, a substantially increased risk for several outcomes remained for women hospitalized for schizophrenia during pregnancy.

The separate analyses of women with schizophrenia, schizophrenia diagnosed before childbirth, and women admitted to hospital for schizophrenia during pregnancy, indicate that the time of case ascertainment in relation to childbirth is of importance. McNeil (1991) concluded that findings on the relationship between severity of maternal mental illness and obstetric complications were inconclusive, but with a tendency for greater severity of mental illness to be related to more obstetric complications. A second explanation for the differences between the three categories of women with schizophrenia could be that the prevalence of other risk factors might vary. In our study, for all women with schizophrenia and for women with

schizophrenia before childbirth, the increased risk of adverse pregnancy outcomes were mainly mediated by the measured covariates, indicating that lifestyle influences the risks. However, among women who experienced a psychotic episode when pregnant, the risks for low birth weight and preterm delivery were still markedly higher even after controlling for maternal social disadvantage and excessive smoking. Women treated for schizophrenia during pregnancy may more often consume antipsychotics (Lanczik *et al.*, 1998), be more likely to continue to smoke during pregnancy, or attend antenatal care clinics less frequently than women without the disease or women without an acute psychosis during pregnancy (Bagedahl-Strindlund, 1986; Bennedsen *et al.*, 2001b; Wrede *et al.*, 1980) and these differences could also explain the differences in risk between the groups.

We found differences in the pattern of the associations between schizophrenia and the different adverse pregnancy outcomes in the offspring, suggesting different underlying mechanisms for the various outcomes. We found maternal, parental, and indications of a genetic effect for low birth weight. The risk for preterm delivery was solely due to maternal effects, whereas the risk of delivering a SGA infant was mostly due to a parental effect. For infant death, parental effects (including paternal effects) were important, even after controlling for covariates.

A recent meta-analysis showed that the risk for infant mortality was higher than expected among parents with schizophrenia (Webb *et al.*, 2005), but could not partition the risk in maternal and paternal components. In paper III, we found a substantial paternal effect for infant death even after adjustment for covariates. The significantly increased risks for both mothers and fathers with schizophrenia suggest that, in addition to maternal behaviour, also paternal factors are of importance. Moreover, in line with Bennedsen *et al.* (2001), we found an increased risk for postneonatal death among infants with a mother or father with schizophrenia, possibly indicating that parental maltreatment is of importance (Bennedsen *et al.*, 2001a).

We could also show that offspring to fathers with schizophrenia has increased risks for low birth weight, and being SGA. Our study is much larger than previous studies, and we find it likely that paternal schizophrenia also confers a risk for adverse pregnancy outcomes. Nonetheless, since the risks were attenuated when covariates were included in the models, we agree with Sacker et al. (1996), that adverse pregnancy outcomes

were not a consequence of schizophrenia in the parents, but rather a consequence of the adverse socio-environmental conditions.

Previous studies have shown that adverse pregnancy outcomes are risk factors for later development of schizophrenia (Cannon *et al.*, 2002). Moreover, both schizophrenia (Sullivan *et al.*, 2003) as well as most adverse pregnancy outcomes, e.g., birth weight (Clausson *et al.*, 2000) are heritable traits. Based on these findings, we set out to investigate whether schizophrenia and adverse pregnancy outcomes share etiology. Our results indicate that the risk for low birth weight among offspring to schizophrenic parents may partly contribute to the schizophrenia liability in the offspring, and thus contribute to part of the heritability explained for schizophrenia. However, the increased risks for parental siblings were low and the results were not unambiguous. It is also possible that the covariates with heritable components, e.g., smoking, mediate these genetic effects. The tendency of a common familial factor found in the association between schizophrenia and low birth weight might, besides common genetic effects, also be due to shared environmental factors in the family. The shared environmental component for schizophrenia is relatively low (Sullivan *et al.*, 2003), and the common familial factor is therefore more likely to be due to genetic components rather than shared environmental components.

Schizophrenia as a consequence of adverse pregnancy outcomes

In the fourth study in this thesis we found that low birth weight is associated with later development of schizophrenia. Further, the results from the cohort analyses as well as the within pair analyses showed that measures of fetal growth restriction were associated with schizophrenia. The results from the cohort study were supported by the analyses within twin pairs discordant for disease, indicating that the association between fetal growth restriction and the risk of schizophrenia is independent of familial factors. Other studies on twins discordant for schizophrenia have shown conflicting results (Gottesman & Shields, 1976; Kunugi *et al.*, 2003; McNeil *et al.*, 1994; Onstad *et al.*, 1992; Pollin, 1968; Torrey, 1977). However, most of these twin studies obtained their obstetric data from maternal or other relatives' recall rather than hospital records (Kunugi *et al.*, 2001). We categorized birth weight and regarded the lowest quartile as low birth weight babies. The same principle was used regarding small head circumference. This is in line with Cannon *et al.* (2002), who argue that arbitrary

cutoffs in birth weight may account for the heterogeneity in the association between low birth weight and schizophrenia in previous studies.

An association between small head circumference at birth and the later development of schizophrenia, after controlling for gestational age, has been shown in previous studies (Cantor-Graae *et al.*, 1998; Kunugi *et al.*, 1996; McNeil *et al.*, 2000). We replicated this finding in the cohort study and the within twin pair analyses confirmed the results. Moreover, the within pair analyses showed that the association between small head circumference and schizophrenia was not confounded by familial factors.

One of the most consistent findings in the search for risk factors for schizophrenia has been that asphyxia at birth predisposes to schizophrenia in adult life (Dalman *et al.*, 1999; Hultman *et al.*, 1999; Jones *et al.*, 1998). The twins included in paper IV were born between 1926 and 1974, when neonatal complications of the infant were classified differently from today. Signs of asphyxia at birth, weakness after delivery and the need for a child to remain under care after birth were noted with the diagnosis 'congenital debility'. We used this diagnosis, since we were unable to use Apgar score, which is the current standardized assessment of asphyctic signs after delivery. We found that infants diagnosed with congenital debility had an increased risk for schizophrenia. Thus, it is possible that early signs of weakness, in addition to low birth weight, small head circumference and/or fetal growth restriction, are important predictors of later development of schizophrenia.

The role of adverse pregnancy outcomes in schizophrenia

Similar to others (Bennedsen *et al.*, 1999; Jablensky *et al.*, 2005; Webb *et al.*, 2005), the studies included in this thesis have shown that schizophrenia in mother is a risk factor for adverse pregnancy outcomes. Further, women admitted to hospital for schizophrenia during pregnancy is at the highest risk, suggesting that women treated in hospital during pregnancy do not obtain benefits in terms of improved pregnancy outcome. It is possible that they do not receive the appropriate surveillance and antenatal care, or that this care is not helpful. Further understanding of why these women are not helped by the antenatal care and how the maternal health care system more efficiently can provide intervention is crucial.

The population-based studies in this thesis allowed us to take a first step in the important task of elucidating the causal mechanisms underlying the increased risks for adverse pregnancy outcomes among women with schizophrenia. Previous studies have emphasized the importance of maternal disadvantageous lifestyle as a possible explanation of the association (Bennedsen, 1998). We could show that maternal smoking, low socio-economic status, and other confounding variables account for a large part of the increased risks. However, since also offspring to fathers with schizophrenia had increased risks for adverse pregnancy outcomes, other factors than maternal behaviour must play a role.

A particularly interesting finding was that a parental factor contributed to the risk for infant death, that is, offspring to both mothers and fathers with schizophrenia had an increased risk for death during the postneonatal period. Parents with schizophrenia may have a reduced ability to read infants' cues, and weak social support networks may complicate good caring (Hultman *et al.*, 1997b; Poole *et al.*, 2000). Our results prompt for better surveillance of these families at risk.

Schizophrenia is considered to be under a high degree of genetic influence and the heritability has been estimated to around 80 per cent (Sullivan *et al.*, 2003). Furthermore, we and others have shown that obstetric complications increase the risk for later development of schizophrenia (Geddes & Lawrie, 1995; Hultman *et al.*, 1997a; McNeil, 1995; Nilsson *et al.*, 2005). The results in paper III indicated that part of the vulnerability for schizophrenia may share susceptibility genes with low birth weight. If this is true, it raises the question whether part of the heritable effects for schizophrenia are mediated by birth weight. On the other hand, in paper IV, the within pair analyses suggested that the association between fetal growth restriction and risk of schizophrenia is probably independent of familial factors. Even though the precision of this study was limited, it is not likely that birth weight explains a major part of the heritability for schizophrenia.

Previous research has not been able to show whether the excess risk of obstetric complications in individuals who later develop schizophrenia reflect an early neurodevelopmental impairment or a later insult in connection to the delivery (Waddington *et al.*, 1999). Congenital malformations and minor physical anomalies have traditionally been regarded as markers of early neurodevelopmental impairment.

These anomalies occur in first and/or early second trimester (Waddington *et al.*, 1999). However, it is unclear if obstetric complications (above all low birth weight/fetal growth restriction) often used in studies of risk factors for schizophrenia are markers of a neurodevelopmental impairment. Paper IV supports the hypothesis of schizophrenia as a neurodevelopmental disorder in two ways. First, the differences in neonatal size have previously been thought of as determined by differences in fetal growth occurring only in the second half of the pregnancy. However, a recent study found that larger neonates have higher growth velocity during early prenatal life and that the difference in growth velocity between smaller and larger neonates is apparent between month four and five. In the 22 week of gestation the growth velocity of fetal weight was found to be higher among larger compared to smaller neonates. The same was true for head circumference, the differences in growth velocity for head circumference is higher for larger neonates compared to smaller even earlier in gestation (18 weeks) (Milani *et al.*, 2005). We found increased risks for schizophrenia for both infants with low birth weight and for infants with small head circumference, indicating that a chronic fetal growth restriction starting relatively early in pregnancy may be the reason for later development of schizophrenia. Second, in our study of discordant twins it was more common that the smaller twin in the pair later developed schizophrenia. Thus, fetal growth restriction is associated with schizophrenia independently of familial factors, supporting the presence of a direct effect on the smaller twin during pregnancy.

Early risk factors represent some of the most challenging targets of psychiatric epidemiology. We have examined some features of adverse pregnancy outcomes within large cohorts and with modern epidemiological methods. Register-based family studies have proven to be fertile in the search for new approaches to elucidate patterns of causality. However, research on early exposures related to psychiatric disorders is still in its infancy and no consensus has yet been reached concerning the interpretation of the excess of deviations from normal conditions and possible mechanisms involved. There are several issues that need to be considered for the future. Even if we have non-biased and prospectively collected information from birth records or registers, data are often not specific enough to characterise the exposure and the timing during the fetal period in enough detail. Thus, there is evidence of some kind of non-optimal environmental influence, but the variables are ‘proxies’ of a range of possible biological pathways. Another issue concerns specificity of

findings. It is not likely that the increased risk for adverse pregnancy outcomes is unique for schizophrenia. The same exposures, especially those related to fetal growth, seem to be related to a range of psychiatric disorders (schizophrenia, affective disorder, infantile autism, anorexia nervosa) (Cnattingius *et al.*, 1999; Hultman *et al.*, 2002; Hultman *et al.*, 1999). Non-specificity might, however, increase and challenge rather than decrease the relevance of pre- and perinatal risk factors with the goal to find common causes.

Conclusions

- Genetic effects are of importance for the development of pre-eclampsia as well as for the development of gestational hypertension.
- Pre-eclampsia and gestational hypertension may share a common genetic etiology.
- Schizophrenia in mother and/or father increases the risk for adverse pregnancy outcomes in the offspring. Women admitted to hospital for schizophrenia during pregnancy have the highest risks for adverse pregnancy outcomes.
- The increased risks of adverse pregnancy outcomes among parents with schizophrenia are primarily explained by confounding factors such as smoking, low education, and single motherhood.
- The increased risk for infant death among offspring to parents with schizophrenia can in part be explained by parental behavior.
- Both low birth weight and fetal growth restriction seem to influence susceptibility to schizophrenia.
- Fetal growth restriction seems to be associated with risk of schizophrenia independent of familial factors.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to everyone who has contributed to this thesis. In particular, I would like to thank:

Paul Lichtenstein, my principle supervisor. For sharing your expertise and knowledge in genetic epidemiology. For your support, listening, rapid responses and for always being available. And also, for your enormous ability to inspire me even in times when I have doubted my abilities, I always leave our meetings with new energy and greater confidence in myself.

Sven Cnattingius, my co-supervisor, for sharing your profound knowledge in reproductive epidemiology in an always generous and straightforward way. Your friendly support, help and advice has meant a lot to me!

Christina Hultman, my co-supervisor, for your never-ending and enthusiastic encouragements. Our discussions about the mysteries of schizophrenia and your deep knowledge and great interest in psychiatric epidemiology have really inspired me!

Petra Otterblad Olausson, my co-supervisor and boss at the Centre for Epidemiology, for encouragement and support, both to complete my thesis and in everyday work at the EpC.

My co-authors, for expertise and essential contributions; *Helena Salonen Ros* in paper I and *Robin M. Murray* in paper II. *Gabriella Stålberg*, co-author in paper IV, especially for your hard and thorough work collecting data for the study and for many friendly conversations.

Nancy Pedersen, the new head of the Department of Medical Epidemiology and Biostatistics, thank you for all the years at the Twin Register!

Hans-Olov Adami, the creator and former head of the department, for giving me the opportunity to perform my thesis at the best of departments.

Måns Rosén, head of the Centre for Epidemiology, for giving me the opportunity to finish my thesis and for creating a wonderful working atmosphere at EpC.

Catherine Tuvblad, for endless discussions about all and nothing, for your great sense of humour and for all the fun we have had during the years and *Jenny Carlsson, Pia Svedberg, Camilla Björk, Jurgita Narusyte, Anastasia Iliadou, Anna Svensson*, for discussions, inspiration, and all the fun!! *Niklas Bergvall, Susanne Buchmayer, Eva Carlström, Alexandra Ekman, Mats Forsman, Sanna Gustafson, Lena George, Andreas Jacks, Catarina Jansson, Anna Johansson, Sofie Johansson, Åsa Klint, Kenji Kato, Henrik Larsson, Anna Lindam, Kerstin Linderholm, Linda Lindström, Lennart Martinsson, Michaela Prochazka, Denny Rönngren, Pär Sparén, Johan Söderberg, Mats Talbäck* for support, interesting conversations, and making everyday work pleasant!

Monica Rundgren and *Gunilla Sonnebring*. Monica, for your invaluable help with various things and patience with all my questions. Gunilla, for small talks and for generously, patiently, and kindly helping me with linguistic revisions and other practical matters.

All other former and present PhD-students and co-workers, for making MEB a great place to work at!

Friends and colleagues at the Centre for Epidemiology and especially *Birgitta Ollars, Frida Lundgren,* and *Milla Bennis* for facilitating my work at the EpC during this final year of my PhD-studies.

My friends outside the world of epidemiology. For all the fun and laughter, your endless support, and for always believing in me. You are the best!!

My family, my parents, Eva and Gunnar, for caring, love and support. Min älskade syster Hanna och världens bästa Morfar Karl-Axel för att ni är ni, and Hans for your ”subtle” hints about research.

Fredrik, for love, patience and everything else.

The funding sources that supported this research, *the Swedish Council for Social Research, the Swedish Council for Working Life and Social Research, the Swedish Research Council, and the Swedish Cancer Society.*

REFERENCES

Aberg, A. & Lindmark, G. (1992) Competence and compliance in antenatal care. Experience from Sweden. *Int J Technol Assess Health Care*, *8*, 20-24.

Arngrimsson, R., Bjornsson, H. & Geirsson, R. T. (1995) Analysis of different inheritance patterns in preeclampsia/eclampsia syndrome. *Hypertension Pregn*, *14*, 27-38.

Arntzen, A., Samuelsen, S. O., Bakketeig, L. S. & Stoltenberg, C. (2004) Socioeconomic status and risk of infant death. A population-based study of trends in Norway, 1967-1998. *Int J Epidemiol*, *33*, 279-288.

Bagedahl-Strindlund, M. (1986) Mentally ill mothers and their children. An epidemiological study of antenatal care consumption, obstetric conditions, and neonatal health. *Acta Psychiatr Scand*, *74*, 32-40.

Barker, D. J. (1998) In utero programming of chronic disease. *Clin Sci (Lond)*, *95*, 115-128.

---- (2002) Fetal programming of coronary heart disease. *Trends Endocrinol Metab*, *13*, 364-368.

Barton, J. R., O'Brien J, M., Bergauer, N. K., Jacques, D. L. & Sibai, B. M. (2001) Mild gestational hypertension remote from term: progression and outcome. *Am J Obstet Gynecol*, *184*, 979-983.

Bennedsen, B. E. (1998) Adverse pregnancy outcome in schizophrenic women: occurrence and risk factors. *Schizophr Res*, *33*, 1-26.

Bennedsen, B. E., Mortensen, P. B., Olesen, A. V. & Henriksen, T. B. (1999) Preterm birth and intra-uterine growth retardation among children of women with schizophrenia. *Br J Psychiatry*, *175*, 239-245.

---- (2001a) Congenital malformations, stillbirths, and infant deaths among children of women with schizophrenia. *Arch Gen Psychiatry*, *58*, 674-679.

Bennedsen, B. E., Mortensen, P. B., Olesen, A. V., Henriksen, T. B. & Frydenberg, M. (2001b) Obstetric complications in women with schizophrenia. *Schizophr Res*, *47*, 167-175.

Berkowitz, G. S. & Papiernik, E. (1993) Epidemiology of preterm birth. *Epidemiol Rev*, *15*, 414-443.

Bosio, P. M., McKenna, P. J., Conroy, R. & O'Herlihy, C. (1999) Maternal central hemodynamics in hypertensive disorders of pregnancy. *Obstet Gynecol*, *94*, 978-984.

Broome, M. R., Woolley, J. B., Tabraham, P., Johns, L. C., Bramon, E., Murray, G. K., Pariante, C., McGuire, P. K. & Murray, R. M. (2005) What causes the onset of psychosis? *Schizophr Res*, *79*, 23-34.

Buchbinder, A., Sibai, B. M., Caritis, S., Macpherson, C., Hauth, J., Lindheimer, M. D., Klebanoff, M., Vandorsten, P., Landon, M., Paul, R., Miodovnik, M., Meis, P. & Thurnau, G. (2002) Adverse perinatal outcomes are significantly higher in severe gestational hypertension than in mild preeclampsia. *Am J Obstet Gynecol*, *186*, 66-71.

Byrne, M., Browne, R., Mulryan, N., Scully, A., Morris, M., Kinsella, A., Takei, N., McNeil, T., Walsh, D. & O'Callaghan, E. (2000) Labour and delivery complications and schizophrenia. Case-control study using contemporaneous labour ward records. *Br J Psychiatry*, *176*, 531-536.

Campbell, D. M., MacGillivray, I. & Carr-Hill, R. (1985) Pre-eclampsia in second pregnancy. *Br J Obstet Gynaecol*, *92*, 131-140.

Cannon, M., Jones, P. B. & Murray, R. M. (2002) Obstetric complications and schizophrenia: historical and meta-analytic review. *Am J Psychiatry*, *159*, 1080-1092.

Cantor-Graae, E., Ismail, B. & McNeil, T. F. (1998) Neonatal head circumference and related indices of disturbed fetal development in schizophrenic patients. *Schizophr Res*, *32*, 191-199.

Carr, D. B., Epplein, M., Johnson, C. O., Easterling, T. R. & Critchlow, C. W. (2005) A sister's risk: family history as a predictor of preeclampsia. *Am J Obstet Gynecol*, *193*, 965-972.

Centre for Epidemiology (2003) The Swedish Medical Birth Register - A Summary of Content and Quality. In *Research Report*, Available at: http://www.sos.se/fulltext/112/2003-2112-2003/2003-2112-2003.pdf: National Board of Health and Welfare.
---- (2004) Causes of Death 2002. In *Research Report*, Available at: http://www.socialstyrelsen.se/Publicerat/2004/8533/2004-2042-2005.htm: National Board of Health and Welfare.
---- (2005) The Swedish Hospital Discharge Register. Available at: http://www.sos.se/epc/english/ParEng.htm#Publications: The National Board of Health and Welfare.
Chesley, L. C. (1984) History and epidemiology of preeclampsia-eclampsia. *Clin Obstet Gynecol*, *27*, 801-820.
Christensen, K., Wienke, A., Skytthe, A., Holm, N. V., Vaupel, J. W. & Yashin, A. I. (2001) Cardiovascular mortality in twins and the fetal origins hypothesis. *Twin Res*, *4*, 344-349.
Clausson, B., Lichtenstein, P. & Cnattingius, S. (2000) Genetic influence on birthweight and gestational length determined by studies in offspring of twins. *Bjog*, *107*, 375-381.
Cnattingius, S. (2004) The epidemiology of smoking during pregnancy: smoking prevalence, maternal characteristics, and pregnancy outcomes. *Nicotine Tob Res*, *6 Suppl 2*, S125-140.
Cnattingius, S., Hultman, C. M., Dahl, M. & Sparen, P. (1999) Very preterm birth, birth trauma, and the risk of anorexia nervosa among girls. *Arch Gen Psychiatry*, *56*, 634-638.
Cnattingius, S., Mills, J. L., Yuen, J., Eriksson, O. & Salonen, H. (1997) The paradoxical effect of smoking in preeclamptic pregnancies: smoking reduces the incidence but increases the rates of perinatal mortality, abruptio placentae, and intrauterine growth restriction. *Am J Obstet Gynecol*, *177*, 156-161.
Cnattingius, S., Reilly, M., Pawitan, Y. & Lichtenstein, P. (2004) Maternal and fetal genetic factors account for most of familial aggregation of preeclampsia: a population-based Swedish cohort study. *Am J Med Genet A*, *130*, 365-371.
Cooper, D. W. & Liston, W. A. (1979) Genetic control of severe pre-eclampsia. *J Med Genet*, *16*, 409-416.
Dalman, C., Allebeck, P., Cullberg, J., Grunewald, C. & Koster, M. (1999) Obstetric complications and the risk of schizophrenia: a longitudinal study of a national birth cohort. *Arch Gen Psychiatry*, *56*, 234-240.
Dalman, C., Broms, J., Cullberg, J. & Allebeck, P. (2002) Young cases of schizophrenia identified in a national inpatient register--are the diagnoses valid? *Soc Psychiatry Psychiatr Epidemiol*, *37*, 527-531.
de Leon, J., Dadvand, M., Canuso, C., White, A. O., Stanilla, J. K. & Simpson, G. M. (1995) Schizophrenia and smoking: an epidemiological survey in a state hospital. *Am J Psychiatry*, *152*, 453-455.
Diggle, P. J., Liang, K.-Y. & Zeger, S. L. (1994) *Analysis of longitudinal data*: Oxford University Press.
Diwan, A., Castine, M., Pomerleau, C. S., Meador-Woodruff, J. H. & Dalack, G. W. (1998) Differential prevalence of cigarette smoking in patients with schizophrenic vs mood disorders. *Schizophr Res*, *33*, 113-118.
Duckitt, K. & Harrington, D. (2005) Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. *Bmj*, *330*, 565.
Ekholm, B., Ekholm, A., Adolfsson, R., Vares, M., Ösby, U., Sedvall, G. C. & Jönsson, E. G. (2005) Evaluation of diagnostic procedures in Swedish patients with schizophrenia and related psychoses. *Nordic Journal of Psychiatry*, *In press*.
Esplin, M. S., Fausett, M. B., Fraser, A., Kerber, R., Mineau, G., Carrillo, J. & Varner, M. W. (2001) Paternal and maternal components of the predisposition to preeclampsia. *N Engl J Med*, *344*, 867-872.
Fish, B. & Kendler, K. S. (2005) Abnormal infant neurodevelopment predicts schizophrenia spectrum disorders. *J Child Adolesc Psychopharmacol*, *15*, 348-361.
Geddes, J. R. & Lawrie, S. M. (1995) Obstetric complications and schizophrenia: a meta-analysis. *Br J Psychiatry*, *167*, 786-793.

Goff, D. C., Henderson, D. C. & Amico, E. (1992) Cigarette smoking in schizophrenia: relationship to psychopathology and medication side effects. *Am J Psychiatry*, *149*, 1189-1194.
Goldenberg, R. L., Kirby, R. & Culhane, J. F. (2004) Stillbirth: a review. *J Matern Fetal Neonatal Med*, *16*, 79-94.
Gottesman, II & Shields, J. (1976) A critical review of recent adoption, twin, and family studies of schizophrenia: behavioral genetics perspectives. *Schizophr Bull*, *2*, 360-401.
Hauth, J. C., Ewell, M. G., Levine, R. J., Esterlitz, J. R., Sibai, B., Curet, L. B., Catalano, P. M. & Morris, C. D. (2000) Pregnancy outcomes in healthy nulliparas who developed hypertension. Calcium for Preeclampsia Prevention Study Group. *Obstet Gynecol*, *95*, 24-28.
Hultman, C. M., Ohman, A., Cnattingius, S., Wieselgren, I. M. & Lindstrom, L. H. (1997a) Prenatal and neonatal risk factors for schizophrenia. *Br J Psychiatry*, *170*, 128-133.
Hultman, C. M., Sparen, P. & Cnattingius, S. (2002) Perinatal risk factors for infantile autism. *Epidemiology*, *13*, 417-423.
Hultman, C. M., Sparen, P., Takei, N., Murray, R. M. & Cnattingius, S. (1999) Prenatal and perinatal risk factors for schizophrenia, affective psychosis, and reactive psychosis of early onset: case-control study. *Bmj*, *318*, 421-426.
Hultman, C. M., Wieselgren, I. M. & Ohman, A. (1997b) Relationships between social support, social coping and life events in the relapse of schizophrenic patients. *Scand J Psychol*, *38*, 3-13.
Innes, K. E., Wimsatt, J. H. & McDuffie, R. (2001) Relative glucose tolerance and subsequent development of hypertension in pregnancy. *Obstet Gynecol*, *97*, 905-910.
Jablensky, A. V., Morgan, V., Zubrick, S. R., Bower, C. & Yellachich, L. A. (2005) Pregnancy, delivery, and neonatal complications in a population cohort of women with schizophrenia and major affective disorders. *Am J Psychiatry*, *162*, 79-91.
Jaquet, D., Swaminathan, S., Alexander, G. R., Czernichow, P., Collin, D., Salihu, H. M., Kirby, R. S. & Levy-Marchal, C. (2005) Significant paternal contribution to the risk of small for gestational age. *Bjog*, *112*, 153-159.
Jones, P. B., Rantakallio, P., Hartikainen, A. L., Isohanni, M. & Sipila, P. (1998) Schizophrenia as a long-term outcome of pregnancy, delivery, and perinatal complications: a 28-year follow-up of the 1966 north Finland general population birth cohort. *Am J Psychiatry*, *155*, 355-364.
Kendler, K. S., Pedersen, N. L., Farahmand, B. Y. & Persson, P. G. (1996) The treated incidence of psychotic and affective illness in twins compared with population expectation: a study in the Swedish Twin and Psychiatric Registries. *Psychol Med*, *26*, 1135-1144.
Klaning, U. (1999) Greater occurrence of schizophrenia in dizygotic but not monozygotic twins. Register-based study. *Br J Psychiatry*, *175*, 407-409.
Klaning, U., Pedersen, C. B., Mortensen, P. B., Kyvik, K. O. & Skytthe, A. (2002) A possible association between the genetic predisposition for dizygotic twinning and schizophrenia. *Schizophr Res*, *58*, 31-35.
Kramer, M. S. (1987) Intrauterine growth and gestational duration determinants. *Pediatrics*, *80*, 502-511.
Kristjansson, E., Allebeck, P. & Wistedt, B. (1987) Validity of the diagnosis schizophrenia in a psychiatric inpatient register: A retrospective application of DSM-III criteria on ICD-8 diagnoses in Stockholm county. *Nordisk Psykiatrisk Tidsskrift*, *41*, 229-234.
Kunugi, H., Nanko, S. & Murray, R. M. (2001) Obstetric complications and schizophrenia: prenatal underdevelopment and subsequent neurodevelopmental impairment. *Br J Psychiatry Suppl*, *40*, s25-29.
Kunugi, H., Takei, N., Murray, R. M., Saito, K. & Nanko, S. (1996) Small head circumference at birth in schizophrenia. *Schizophr Res*, *20*, 165-170.
Kunugi, H., Urushibara, T., Murray, R. M., Nanko, S. & Hirose, T. (2003) Prenatal underdevelopment and schizophrenia: a case report of monozygotic twins. *Psychiatry Clin Neurosci*, *57*, 271-274.

Kuritz, S. J., Landis, J. R. & Koch, G. G. (1988) A general overview of Mantel-Haenszel methods: applications and recent developments. *Annual review of public health*, *9*, 123-160.
Lain, K. Y. & Roberts, J. M. (2002) Contemporary concepts of the pathogenesis and management of preeclampsia. *Jama*, *287*, 3183-3186.
Lanczik, M., Knoche, M. & Fritze, J. (1998) [Psychopharmacotherapy during pregnancy and lactation. 1: Pregnancy]. *Nervenarzt*, *69*, 1-9.
Leon, D. A. (2001) The foetal origins of adult disease: interpreting the evidence from twin studies. *Twin Res*, *4*, 321-326.
Lichtenstein, P., De Faire, U., Floderus, B., Svartengren, M., Svedberg, P. & Pedersen, N. L. (2002) The Swedish Twin Registry: a unique resource for clinical, epidemiological and genetic studies. *J Intern Med*, *252*, 184-205.
Lie, R. T., Rasmussen, S., Brunborg, H., Gjessing, H. K., Lie-Nielsen, E. & Irgens, L. M. (1998) Fetal and maternal contributions to risk of pre-eclampsia: population based study. *Bmj*, *316*, 1343-1347.
Loos, R. J., Beunen, G., Fagard, R., Derom, C. & Vlietinck, R. (2001) The influence of zygosity and chorion type on fat distribution in young adult twins consequences for twin studies. *Twin Res*, *4*, 356-364.
Lucassen, A. & Parker, M. (2001) Revealing false paternity: some ethical considerations. *Lancet*, *357*, 1033-1035.
Luginaah, I. N., Lee, K. S., Abernathy, T. J., Sheehan, D. & Webster, G. (1999) Trends and variations in perinatal mortality and low birthweight: the contribution of socio-economic factors. *Can J Public Health*, *90*, 377-381.
Lunde, A. S., Lundeborg, S., Lettenstrom, G. S., Thygesen, L. & Huebner, J. (1980) The person-number systems of Sweden, Norway, Denmark, and Israel. *Vital Health Stat*, *2*, 1-59.
Magnus, P., Gjessing, H. K., Skrondal, A. & Skjaerven, R. (2001) Paternal contribution to birth weight. *J Epidemiol Community Health*, *55*, 873-877.
Marsal, K., Persson, P. H., Larsen, T., Lilja, H., Selbing, A. & Sultan, B. (1996) Intrauterine growth curves based on ultrasonically estimated foetal weights. *Acta Paediatr*, *85*, 843-848.
McNeil, T. F. (1991) Obstetric complications in schizophrenic parents. *Schizophr Res*, *5*, 89-101.
---- (1995) Perinatal risk factors and schizophrenia: selective review and methodological concerns. *Epidemiol Rev*, *17*, 107-112.
McNeil, T. F., Cantor-Graae, E. & Ismail, B. (2000) Obstetric complications and congenital malformation in schizophrenia. *Brain Res Brain Res Rev*, *31*, 166-178.
McNeil, T. F., Cantor-Graae, E., Torrey, E. F., Sjostrom, K., Bowler, A., Taylor, E., Rawlings, R. & Higgins, E. S. (1994) Obstetric complications in histories of monozygotic twins discordant and concordant for schizophrenia. *Acta Psychiatr Scand*, *89*, 196-204.
McNeil, T. F., Kaij, L. & Malmquist-Larsson, A. (1983) Pregnant women with nonorganic psychosis: life situation and experience of pregnancy. *Acta Psychiatr Scand*, *68*, 445-457.
Milani, S., Bossi, A., Bertino, E., di Battista, E., Coscia, A., Aicardi, G., Fabris, C. & Benso, L. (2005) Differences in size at birth are determined by differences in growth velocity during early prenatal life. *Pediatr Res*, *57*, 205-210.
Mogren, I., Hogberg, U., Winkvist, A. & Stenlund, H. (1999) Familial occurrence of preeclampsia. *Epidemiology*, *10*, 518-522.
Mueser, K. T. & McGurk, S. R. (2004) Schizophrenia. *Lancet*, *363*, 2063-2072.
Naeye, R. L. (1981) Maternal blood pressure and fetal growth. *Am J Obstet Gynecol*, *141*, 780-787.
Neale, M. & Cardon, L. (1992) *Methodology for genetic studies of twins and families*. Dordrecht: Kluwer.
Neale, M. C., Boker, S. M., Xie, G. & Maes, H. H. (1999) *Mx:statistical modeling* (5th edition edn). Richmond: Virginia Commonwealth University.
Nilsson, E., Stalberg, G., Lichtenstein, P., Cnattingius, S., Olausson, P. O. & Hultman, C. M. (2005) Fetal growth restriction and schizophrenia: a Swedish twin study. *Twin Res Hum Genet*, *8*, 402-408.

Nordstrom, M. L., Cnattingius, S. & Haglund, B. (1993) Social differences in Swedish infant mortality by cause of death, 1983 to 1986. *Am J Public Health*, *83*, 26-30.
Onstad, S., Skre, I., Torgersen, S. & Kringlen, E. (1992) Birthweight and obstetric complications in schizophrenic twins. *Acta Psychiatr Scand*, *85*, 70-73.
Ozanne, S. E., Fernandez-Twinn, D. & Hales, C. N. (2004) Fetal growth and adult diseases. *Semin Perinatol*, *28*, 81-87.
Page, E. W. & Christianson, R. (1976) Influence of blood pressure changes with and without proteinuria upon outcome of pregnancy. *Am J Obstet Gynecol*, *126*, 821-833.
Pietilainen, K. H., Kaprio, J., Rissanen, A., Winter, T., Rimpela, A., Viken, R. J. & Rose, R. J. (1999) Distribution and heritability of BMI in Finnish adolescents aged 16y and 17y: a study of 4884 twins and 2509 singletons. *Int J Obes Relat Metab Disord*, *23*, 107-115.
Plomin, R., DeFries, J. C., McClearn, G. E. & McGuffin, P. (2001) *Behavioral Genetics* (Fourth edition edn). New York: Worth Publishers.
Pollin, W., Stabenau, J. R. (1968) Biological, psychological and historical differences in a series of monozygotic twins discordant for schizophrenia. In *The transmission of schizophrenia* (ed D. Rosenthal, Kety, S.,S.), pp. 317-332. London: Pergamon.
Poole, J. H., Tobias, F. C. & Vinogradov, S. (2000) The functional relevance of affect recognition errors in schizophrenia [In Process Citation]. *J Int Neuropsychol Soc*, *6*, 649-658.
Randhawa, R. & Cohen, P. (2005) The role of the insulin-like growth factor system in prenatal growth. *Mol Genet Metab*, *86*, 84-90.
Redman, C. W. & Sargent, I. L. (2005) Latest advances in understanding preeclampsia. *Science*, *308*, 1592-1594.
Roberts, J. M. & Cooper, D. W. (2001a) Pathogenesis and genetics of pre-eclampsia. *Lancet*, *357*, 53-56.
---- (2001b) Pathogenesis and genetics of pre-eclampsia. *Lancet*, *357*, 53-56.
Roberts, J. M. & Lain, K. Y. (2002) Recent Insights into the pathogenesis of pre-eclampsia. *Placenta*, *23*, 359-372.
Roberts, J. M. & Redman, C. W. (1993) Pre-eclampsia: more than pregnancy-induced hypertension. *Lancet*, *341*, 1447-1451.
Ros, H. S., Cnattingius, S. & Lipworth, L. (1998) Comparison of risk factors for preeclampsia and gestational hypertension in a population-based cohort study. *Am J Epidemiol*, *147*, 1062-1070.
Sacker, A., Done, D. J. & Crow, T. J. (1996) Obstetric complications in children born to parents with schizophrenia: a meta-analysis of case-control studies. *Psychol Med*, *26*, 279-287.
Sallout, B. & Walker, M. (2003) The fetal origin of adult diseases. *J Obstet Gynaecol*, *23*, 555-560.
Salonen Ros, H., Lichtenstein, P., Lipworth, L. & Cnattingius, S. (2000) Genetic effects on the liability of developing pre-eclampsia and gestational hypertension. *Am J Med Genet*, *91*, 256-260.
Sexton, M. & Hebel, J. R. (1984) A clinical trial of change in maternal smoking and its effect on birth weight. *Jama*, *251*, 911-915.
Sharp, W. S., Gottesman, R. F., Greenstein, D. K., Ebens, C. L., Rapoport, J. L. & Castellanos, F. X. (2003) Monozygotic twins discordant for attention-deficit/hyperactivity disorder: ascertainment and clinical characteristics. *J Am Acad Child Adolesc Psychiatry*, *42*, 93-97.
Sibai, B., Dekker, G. & Kupferminc, M. (2005) Pre-eclampsia. *Lancet*, *365*, 785-799.
Skjaerven, R., Vatten, L. J., Wilcox, A. J., Ronning, T., Irgens, L. M. & Lie, R. T. (2005) Recurrence of pre-eclampsia across generations: exploring fetal and maternal genetic components in a population based cohort. *Bmj*, *331*, 877.
Statistics Sweden (2001a) Educational attainment of the population 2001-01-01. In *Official statistics of Sweden. UF 37 SM 0101*, Available at: http://www.scb.se/templates/Publikation____44633.asp.
---- (2001b) The Multi-Generation Register. Örebro: Statistics Sweden.

Stephansson, O., Dickman, P. W., Johansson, A. L. & Cnattingius, S. (2001) The influence of socioeconomic status on stillbirth risk in Sweden. *Int J Epidemiol*, *30*, 1296-1301.
Sullivan, P. F., Kendler, K. S. & Neale, M. C. (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry*, *60*, 1187-1192.
Sutherland, A., Cooper, D. W., Howie, P. W., Liston, W. A. & MacGillivray, I. (1981) The indicence of severe pre-eclampsia amongst mothers and mothers-in- law of pre-eclamptics and controls. *Br J Obstet Gynaecol*, *88*, 785-791.
Thadhani, R., Ecker, J. L., Kettyle, E., Sandler, L. & Frigoletto, F. D. (2001) Pulse pressure and risk of preeclampsia: a prospective study. *Obstet Gynecol*, *97*, 515-520.
Thadhani, R., Stampfer, M. J., Hunter, D. J., Manson, J. E., Solomon, C. G. & Curhan, G. C. (1999) High body mass index and hypercholesterolemia: risk of hypertensive disorders of pregnancy. *Obstet Gynecol*, *94*, 543-550.
Thornton, J. G. & Macdonald, A. M. (1999) Twin mothers, pregnancy hypertension and pre-eclampsia. *Br J Obstet Gynaecol*, *106*, 570-575.
Torrey, E. F. (1977) Birth weights, perinatal insults, and HLA types: return to "original din". *Schizophr Bull*, *3*, 347-351.
Treloar, S. A., Cooper, D. W., Brennecke, S. P., Grehan, M. M. & Martin, N. G. (2001) An Australian twin study of the genetic basis of preeclampsia and eclampsia. *Am J Obstet Gynecol*, *184*, 374-381.
Waddington, J. L., Lane, A., Larkin, C. & O'Callaghan, E. (1999) The neurodevelopmental basis of schizophrenia: clinical clues from cerebro-craniofacial dysmorphogenesis, and the roots of a lifetime trajectory of disease. *Biol Psychiatry*, *46*, 31-39.
Wang, J. X., Knottnerus, A. M., Schuit, G., Norman, R. J., Chan, A. & Dekker, G. A. (2002) Surgically obtained sperm, and risk of gestational hypertension and pre-eclampsia. *Lancet*, *359*, 673-674.
Webb, R., Abel, K., Pickles, A. & Appleby, L. (2005) Mortality in offspring of parents with psychotic disorders: a critical review and meta-analysis. *Am J Psychiatry*, *162*, 1045-1056.
Weinberger, D. R. (1987) Implications of normal brain development for the pathogenesis of schizophrenia. *Arch Gen Psychiatry*, *44*, 660-669.
Wen, S. W., Smith, G., Yang, Q. & Walker, M. (2004) Epidemiology of preterm birth and neonatal outcome. *Semin Fetal Neonatal Med*, *9*, 429-435.
World Health Organization (1967) *Swedish version of International Classification of Diseases (ICD-8), 1965 (Klassifikation av sjukdomar 1968. Stockholm, Socialstyrelsen, 1968)*. Geneva: World Health Organization.
---- (1977) *Swedish version of International Classification of Diseases (ICD-9), 1975 (Klassifikation av sjukdomar 1987. Stockholm, Socialstyrelsen, 1986)*. Geneva: World Health Organization.
---- (1992) *Swedish version of International Classification of Diseases (ICD-10), 1997 (Klassifikation av sjukdomar och hälsoproblem 1997. Stockholm, Socialstyrelsen, 1996)*. Geneva: World Health Organization.
Wrede, G., Mednick, S. A., Huttunen, M. O. & Nilsson, C. G. (1980) Pregnancy and delivery complications in the births of an unselected series of Finnish children with schizophrenic mothers. *Acta Psychiatr Scand*, *62*, 369-381.