Fever and infections in pregnancy and neurodevelopmental impairment in the child

PhD thesis

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2017

To be presented with the permission of the Faculty of Health Sciences of the University of Southern Denmark for public examination on February 24th 2017, 14.00-16.00, Auditorium, University of Southern Denmark, Niels Bohrs Vej 9, 6700 Esbjerg, Denmark.

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On the basis of the work presented in this thesis, Julie received the *Esbjerg University Prize* on November 30th 2016.

Cover by: Cecilie Hedelund Werenberg, designer

List of original contributions

The present thesis is based on the following papers.

Paper 1: Dreier JW, Andersen AMN, Berg-Beckhoff G. Systematic Review and Meta-analyses: Fever in Pregnancy and Health Impacts in the Offspring. *Pediatrics*. 2014, 133(3): e674-e688.

Paper 2: Dreier JW, Andersen AMN, Hvolby A, Garne E, Andersen PK, Berg-Beckhoff G. Fever and infections in pregnancy and risk of attention deficit/hyperactivity disorder in the offspring. *Journal of Child Psychology and Psychiatry*. 2016, 57(4): 540-548.

Paper 3: Dreier JW, Berg-Beckhoff G, Andersen PK, Andersen AMN. Prenatal exposure to fever and infections and academic performance. A multilevel analysis. *American Journal of Epidemiology*. Accepted 03/08/2016.

Paper 4: Dreier JW, Berg-Beckhoff G, Andersen AMN, Susser E, Nordentoft M, Strandberg-Larsen. Fever and infections during pregnancy and psychosis-like experiences in the offspring at age 11. A prospective study within the Danish National Birth Cohort. *Submitted*

Acknowledgements

Julie Werenberg Dreier

Esbjerg, November 2016

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Abbreviations

ADHD	Attention deficit/hyperactivity disorder
ALSPAC	Avon Longitudinal Study of Parents and their Children
CNS	Central Nervous System
CI	Confidence Interval
DAG	Directed Acyclic Graph
DNBC	Danish National Birth Cohort
DOHaD	Developmental Origins of Health and Disease
EPA	Estimated Pupil Ability
HR	Hazard ratio
ICD-10	International Statistical Classification of Diseases and Related Health Problems, 10th revision
OR	Odds ratio
RRR	Relative risk ratio
SD	Standard deviation
TORCH-infections	Toxoplasmosis, Others, Rubella, Cytomegalovirus, Herpes
WHO	World Health Organization

Summary

Background

Neurodevelopmental disorders are among the most common causes of disability in school-aged children, and are believed to be a consequence of abnormal or disrupted brain development in early life. Maternal exposure to fever and common infections in pregnancy may interfere with fetal brain development, and have been linked to some neurodevelopmental outcomes, such as cerebral palsy, autism spectrum disorders, and psychotic disorders, but it remains less clear whether such adverse effects extend to a broader range of outcomes, and in particular to milder neurodevelopment impairment.

Aim

The overall aim of this thesis has consequently been to examine whether neurodevelopmental impairment in school-aged children is associated with maternal exposure to fever, and to some extent also common infections in pregnancy. Four papers were included in this thesis, and have specifically sought to establish if there is evidence that maternal fever in pregnancy is associated with health risks in the child, and to examine the association between fever and common infections in pregnancy and three selected neurodevelopmental outcomes in the child (attention deficit/hyperactivity disorder (ADHD), impaired academic performance, and sub-clinical psychosis-like symptoms).

Methods

A systematic literature review and several meta-analyses were conducted as an initial step to summarize available literature on health outcomes in the offspring following maternal fever in pregnancy. Next, three sub-studies addressing ADHD, academic performance and psychosis like experiences, respectively, were carried out within the Danish National Birth Cohort (DNBC). The DNBC is a nationwide birth cohort, which consists of approximately 100.000 mother-child dyads enrolled during 1996-2002. Information on maternal exposure to fever and a range of common infections (genitourinary infections, prolonged cough, diarrhea, persistent viral infections, and influenza-like illness) was obtained from two interviews that were scheduled to take place at approximately pregnancy week 12 and 30. Cases of ADHD were identified using patient discharge- and prescription registries, academic performance was measured using language and math assessments from the Danish National Test Program (2010-2013), and psychosis-like experiences were reported by the children themselves in the 11-year follow-up of the cohort. A variety of statistical methods, such as cox regression, hierarchical linear regression, multinomial logistic regression, and inverse probability weighting were used.

Results

In the systematic literature review, substantial evidence was found to support the contention that maternal fever in pregnancy may negatively affect offspring health. The strongest and most consistent evidence

was available for neural tube defects, in which a meta-analysis suggested a nearly 3-fold increased risk with first-trimester fever exposure. The harmful effects of maternal fever did also seem to cover longer-term developmental outcomes in the child, although for many of the outcomes the evidence was insufficient to judge any association with certainty. In the first study conducted within the DNBC, ADHD occurrence in the child was generally not associated with overall maternal exposure to fever and a range of common infections in pregnancy. Maternal genitourinary infections during pregnancy were, however, associated with a slightly elevated rate of ADHD in the child, and the timing analyses suggested that this association seemed to be limited to and stronger for third-trimester exposure. In the next study, neither prenatal exposure to fever nor any of the examined infections (genitourinary infections, respiratory tract infections, and diarrhea) were associated with academic performance during childhood and early adolescence. These findings were consistent for different types of academic performance (language and math) and for different timings of exposure. In the final study of psychosis-like experiences, maternal fever and common infections generally showed small or no associations with subsequent psychosis-like symptoms in the child. A stronger association was found for influenza-like illness under an a priori definition, but these findings could not be replicated under alternative definitions.

Conclusions

The findings presented in this thesis suggest that while fever in early pregnancy may increase the risk of more pronounced brain defects, prenatal exposure to fever and a range of common infections is generally not or only marginally associated with milder neurodevelopmental impairment in school-aged children, as measured by ADHD, academic performance and psychosis-like experiences. A few associations are demonstrated, but they are either relatively weak or related to rare outcomes, suggesting that the overall risk of a child suffering from these neurodevelopmental consequences remains low, even if the mother is exposed while being pregnant.

Sammenfatning

Baggrund

Neuro-udviklingsmæssige lidelser omfatter en bred gruppe af sygdomme såsom cerebral parese, psykotiske lidelser, autisme spektrum forstyrrelser, attention deficit/hyperactivity disorder (ADHD), tics, indlæringsproblemer og mange flere, og er blandt de hyppigste årsager til funktionsnedsættelse blandt børn i skolealderen. Fælles for disse lidelser er, at de menes at være en konsekvens af forstyrrelser i hjernens udvikling tidligt i livet. Feber og infektioner under graviditeten kan påvirke hjerneudviklingen hos fostret og er blevet sat i forbindelse med nogle af de mere alvorlige og gennemgribende forstyrrelser, såsom autisme og skizofreni. Det er imidlertid uklart hvorvidt de skadelige virkninger af feber og infektioner under graviditeten også omfatter en bredere gruppe af neuro-udviklingsmæssige lidelser, herunder især de mildere og mindre udtalte.

Formål

Det overordnede formål med denne afhandling har derfor været at undersøge om især feber og i nogen udstrækning også almindelig infektioner i graviditeten er associeret med mildere neuro-udviklingsmæssige forstyrrelser hos børn i skolealderen. Afhandlingen omfatter fire artikler, som mere specifikt har søgt at sammenfatte den eksisterende viden om potentielle skadelige virkninger hos barnet, som følge af feber-eksponering under graviditeten, samt at undersøge sammenhængen mellem feber og almindelige infektioner i graviditeten og forekomsten af neuro-udviklingsmæssige forstyrrelser hos barnet, målt ved ADHD, forringet faglig præstation i skolen, og psykose-lignende symptomer.

Metode

Indledningsvist blev den eksisterende viden om sundhedsmæssige konsekvenser hos barnet som følge af moderens eksponering for feber under graviditeten sammenfattet i en systematisk litteraturgennemgang og en række metaanalyser. Med udgangspunkt i de knap 100.000 mødre og børn, som i perioden 1996-2002, blev inkluderet i den nationale fødselskohorte "Bedre Sundhed i Generationer" (BSIG), gennemførtes herefter tre studier med fokus på henholdsvis ADHD, faglig præstation i skolen, og psykose-lignende symptomer. Oplysninger om moderens eksponering for feber og en række infektioner (vaginale og urinvejsinfektioner, langvarig hoste, diarré, kroniske virusinfektioner, samt influenza-lignende sygdom) blev baseret på to interviews, som var planlagt i henholdsvis graviditetsuge 12 og 30. De danske patient- og lægemiddelregistre blev brugt til at identificere hvilke af børnene, der udviklede ADHD, børnenes resultater fra de Nationale Tests i folkeskolen (3020-2013) blev brugt til at vurdere deres faglige præstation i skolen, og psykose-lignende symptomer blev rapporteret af børnene selv i 11-års opfølgningen af kohorten. En række forskellige statistiske metoder blev anvendt i denne afhandling, herunder Cox regression, hierarkisk lineær regression, multinomial logistisk regression samt vægtede analyser.

Resultater

I den systematiske litteraturgennemgang blev der fundet væsentlig evidens for, at feber under graviditeten kan have negative sundhedsmæssige konsekvenser for barnet. Resultaterne vedrørende neuralrørsdefekter var de stærkeste og mest konsekvente, og her viste en metaanalyse, at der var en næsten tre gange forøget risiko for denne slags fødselsdefekt ved feber eksponering i første trimester af graviditeten. De skadelige virkninger af moderens feber syntes også at omfatte mere langsigtede udviklingsmæssige udfald hos barnet, men evidensen var her generelt ikke stærk nok, til kunne drage nogen konklusioner med sikkerhed. I det første studie baseret på børnene fra BSIG, var der overordnet set ikke nogen sammenhæng mellem ADHD hos barnet og feber og en række almindelige infektioner hos moderen under graviditeten. Vaginale- og urinvejsinfektioner var dog forbundet med en let forøget forekomst af ADHD hos børnene, og timing analyserne indikerede at denne sammenhæng var begrænset til og stærkere for eksponering i sidste trimester. I det næste studie, var der ikke nogen sammenhæng mellem hvordan barnet klarede sig i skolen til de Nationale Tests og om moderen havde haft hverken feber eller infektioner (vaginale og urinvejsinfektioner, luftvejsinfektioner, og diarré) under graviditeten. Dette var tilfældet for både præstation i dansk og matematik og uanset hvornår i graviditeten moderen var blevet eksponeret. I det sidste studie, var hverken feber eller en række almindelige infektioner under graviditeten væsentlige risikofaktorer for, at barnet udviklede psykose-lignende symptomer. I en analyse baseret på en a priori definition af influenza-lignende sygdom, var der dog en moderat forøget risiko for at barnet rapporterede psykose-lignende symptomer ved 11-års opfølgningen, men denne sammenhæng kunne ikke genfindes når andre definitioner af influenza-lignende sygdom blev anvendt.

Konklusion

Resultaterne fra denne afhandling viser, at mens feber i første del af graviditeten kan øge risikoen for udtalte skader i hjernens udvikling (neuralrørsdefekter), så er der ikke nogen væsentlig sammenhæng mellem hverken feber eller en række andre almindelige infektioner i graviditeten og mildere neuro-udviklingsmæssige forstyrrelser hos børn i skolealderen, som vurderet ved forekomsten af ADHD, faglig præstation i skolen samt psykose-lignende symptomer. I de tilfælde, hvor der blev fundet nogle sammenhænge, er de enten svage eller relateret til meget sjældne udfald, hvilket betyder, at selv hvis moderen bliver eksponeret under graviditeten, så vil den overordnede risiko for at barnet udvikler disse forstyrrelser forsat være lav.

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Papers

Chapter 1

Introduction

Disturbances of brain development in early stages of life may cause long-term neurodevelopmental deficits in the child.[1] Neurodevelopmental disorders span from rare and very severe disorders to milder and more frequent conditions, but are generally associated with adverse psychological, behavioral and educational consequences. They are estimated to affect approximately one in six children,[2] and are among the most common causes of disability in school-aged children. These conditions constitute a substantial burden not only to the children and families affected, but also to society as they are associated with enormous costs from increased educational-, health-, and social services.[3]

The neurodevelopmental hypothesis was initially developed to explain how schizophrenia could, at least in part, be understood as a consequence of early life conditions.[4-7] One prevailing theory involved prenatal exposure to infections, and in 1988 Mednick and colleagues published the first epidemiological study supporting this theory, by linking influenza exposure in pregnancy to offspring schizophrenia.[8] Since then, other infections and other neurodevelopmental outcomes have been implicated as well, and today it is generally well-established that some infections (rubella, toxoplasmosis, etc.) may indeed cross the placental barrier and interfere with fetal brain development,[9] with the most recent example being the Zika virus.[10,11] The neurodevelopmental consequences of prenatal exposure to more common infections (e.g. urinary tract infections, respiratory infections, etc.), does however remain unclear. These infections are usually not considered to be capable of vertical transmission to the fetus, but are instead hypothesized to exert any potential harm indirectly, for instance through maternal fever. Establishing any potential contribution of such maternal exposures to neurodevelopmental impairment in the child is important in a public health perspective, because fevers and many infections are very common, modifiable and may be targeted for prevention.

1.1 Aims

The overall research question explored in this thesis was consequently whether maternal exposure to fever and common infections during pregnancy may affect fetal brain development and have long-term effects on function in school-aged children. Three outcomes in school-aged children were selected to assess potential neurodevelopmental impairment of these fetal exposures. The studies were based on the children and mothers within the Danish National Birth Cohort, which constitutes one of the largest sources of information on fever exposure in pregnancy. Initially a systematic literature review was conducted to summarize the evidence of health effects in the child following in-utero exposure to maternal fever and to identify the most relevant directions for future research.
The specific aims of the four papers included were:

- To systematically review evidence from epidemiologic studies on adverse health outcomes in the offspring following maternal fever during pregnancy. (**Paper 1**)

- To examine the association between fever and common infections during pregnancy and subsequent occurrence of attention deficit/hyperactivity disorder in the child. (**Paper 2**)
- To examine whether exposure to fever and common infections during pregnancy was associated with academic performance in childhood and early adolescence. (**Paper 3**)
- To examine the association between prenatal exposure to fever and common infections, and child report of psychosis-like experiences at age 11. (**Paper 4**)

In combination, these studies sought to address 1) whether prenatal exposure to fevers and common infections cause neurodevelopmental impairment, 2) whether timing of exposure matter and if there is any periods of increased vulnerability, and 3) what is causing the harm – the infection, the fever, neither or both.

Chapter 2

Background

2.1 Developmental origins of health and disease

The idea that disease risk in adulthood is affected by prenatal and early life events emerged sporadically during the twentieth century.[12] Inspired by the early work of Anders Forsdahl,[13-15] Barker and colleagues, published a number of papers in Lancet[16-18] from 1986 and onwards, that came to be the foundation of the developmental origins of health and disease (DOHaD). In these studies they showed how undernutrition in fetal life affected developmental processes and permanently changed the body's structure and function, and how these changes subsequently were linked to risk of ischemic heart disease in adult life. Initially referred to as Barker's hypothesis, and then later generalized to the DOHaD hypothesis, it was formally proposed, that the intrauterine and infant environment had long-term implications for health and disease.[19,20]

The evidence in support of the DOHaD hypothesis have since then been mounting.[21] Some of the most well-known studies have considered the long-term health effects of in utero exposure to the Dutch famine,[22,23] maternal stress,[24,25] and the 1918 Spanish influenza.[26] The early life origins of health and disease has also become a matter of public concern. For instance, in 2006 the World Health Organization (WHO) concluded in their report on promoting optimal fetal development that "There is now substantial consensus that environmental influences […] during early life can have a fundamental impact on human development, with consequences for biological and social function and behavior throughout the life course. The consequences are substantial, both in terms of the health costs and the loss of human capital, and provide ample justification for the formulation of a concerted global strategy to optimize fetal development."[27] While conditions such as heart disease and diabetes were the focus of many early investigations, another branch within this area of research started in the 21st century to consider long-term effects of abnormal or impaired brain development.[28,29]

2.2 Neurodevelopmental disorders

A neurodevelopmental disorder is characterized by impairments in the functioning of the brain, which affect the child's behavior, memory or ability to learn.[30] Disorders believed to be of neurodevelopmental origin range on a broad continuum from rare and very severe disorders to more frequent and less disabling conditions, encompassing diseases such as cerebral palsy, schizophrenia, intellectual disability (mental retardation), autism spectrum disorders, attention deficit/hyperactivity disorder (ADHD), tic disorders, speech disorders, dyslexia and learning disabilities. The common characteristic of these disorders are that they are believed to be the outcome of some abnormal developmental processes of the brain, in the unborn or very young child.[31]

Brain development in the fetus starts already within a few weeks of conception, and proceeds all the way into adulthood. The brain is however thought to be particularly vulnerable while the child is still within

the womb, as fundamental structures are formed and because of the immense growth. Maternal exposure to harmful agents during this critical period may therefore influence the development of the child's brain and its functionality, which may lead to short as well as longer term disturbances of the child.

While most developing organs are sensitive to harmful exposures primarily in the embryonic period (gestational week 4-10), the central nervous system (CNS) is an exception.[32] **Figure 1** illustrates this extended period of sensitivity, where the child is susceptible to major structural defects in the most part of the first half of the pregnancy (dark blue), and minor structural or functional defects for the remaining part of pregnancy (light blue).
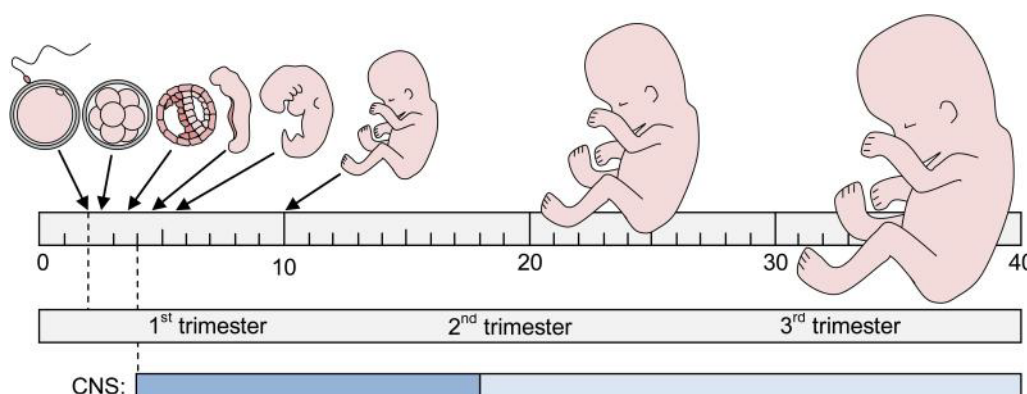
Figure 1: Critical periods of human brain development. The figure illustrates how the central nervous system (CNS) remains sensitive throughout the pregnancy. Maternal exposure to harmful agents may result in major structural defects (dark blue bar), or minor structural and functional defects (light blue bar) depending on the timing. *Adapted from Moore et al.[33], modified by Clift et al.[34]*

Severe neurodevelopmental impairment is estimated to affect approximately 2.5% of the population, while around 15-20% is believed to be affected by less disabling neurodevelopmental disorders.[1,2,30] In the following sections a description of the neurodevelopmental outcomes addressed in this thesis is presented.

2.2.1 Attention deficit/hyperactivity disorder

ADHD is the most common neurodevelopmental disorder in childhood and is characterized by excessive impulsivity, hyperactivity or inattention.[35] The prevalence of ADHD is estimated to be approximately 4-6% in school-aged children.[36,37] Children suffering from ADHD are often more easily distracted, have lower task persistence, and experience difficulties in remaining quietly seated or waiting for their turn, which often leads to academic difficulties as well as peer problems.[38,39] While all of these symptoms may be difficult to distinguish from variations within the normal-range, a diagnosis requires that the symptoms are disruptive and persistent to an extent that is far greater than what is expected for their age.[36] Although

heritability studies of ADHD have shown a strong genetic influence,[40] environmental conditions in early life are increasingly being considered in ADHD etiology as well.[41,42]

2.2.2 Academic performance

Poor academic performance may result from impaired cognition or intelligence, which is also believed to be influenced by the prenatal period of brain development.[43] Substantial damage to the brain during this time may result in intellectual disability,[44-46] but more subtle effects might not be readily observable by parents or physicians. Even though slightly reduced intelligence and impaired academic performance is unlikely to have profound effects on wellbeing of the individual child, the societal costs are substantial due to increases in educational expenses, and reduced economic productivity throughout life.[47,48] For the purpose of this thesis, poor academic performance was considered as a consequence of more subtle neurodevelopmental impairment.

2.2.3 Psychosis-like experiences

Psychosis-like symptoms in childhood, such as hallucinatory experiences and delusional ideas, were in 2000 proposed to foreshadow adult psychoses, in a famous study within the Dunedin Birth Cohort.[49] On the basis of literature linking the prenatal environment to adult psychotic disorders, such symptoms were similarly hypothesized to be affected by impaired neurodevelopmental processes in early life.[50] Psychosis-like symptoms are not clearly defined but may encompass experiences such as hearing voices, seeing people or things that other people cannot see, feelings of being watched or followed, being controlled by external forces, or having supernatural powers. While clinical psychotic disorders only affects a small part of the population, these sub-clinical psychosis-like experiences are expressed much more frequently. In a meta-analysis of population-based studies approximately 17% of children aged 9-12 were estimated to have experienced such symptoms.[51] While many of the children experiencing these symptoms do not go on to develop any mental illness, they are believed to be at increased risk of some psychiatric conditions, poor psycho-social outcomes, and self-harming behavior.[52-55]

2.3 Prenatal exposure to infections

Maternal infection during pregnancy is suggested as one potential explanation for the occurrence of some neurodevelopmental disorders in the offspring. Recently, the potency of prenatal infections has been underscored in Brazil when a sudden surge in cases of microcephaly, a usually rare neurodevelopmental condition, was contributed to maternal Zika virus infection during pregnancy.[10,11] The WHO has declared the Zika virus outbreak a *Public Health Emergency of International Concern*, and has warranted further investigations on the neurodevelopmental consequences of prenatal Zika exposure.[56] This knowledge adds to the well-established evidence concerning neurodevelopmental impairment in the child following maternal exposure in pregnancy to infections belonging to the TORCH complex.[57-59] The TORCH

infections is a group of teratogenic infections and originally referred to *Toxoplasma Gondii*, *Rubella*, *Cytomegalovirus*, and *Herpes Simplex Viruses*. Today, the acronym is used more broadly and encompasses infections such as *Parvovirus B19, Varicella Zoster, Syphilis, Human Papillomavirus* and many more as well.[60,61] Awareness of the harmful fetal consequences of these infections have led to successful preventive strategies (e.g. vaccination against rubella, information about how to avoid toxoplasmosis for cat owners etc.), and the incidence of many of these infections in pregnant women is consequently relatively low in many western countries.[62-64]

Infections, such as influenza, urinary tract infections, vaginal infections, upper respiratory tract infections, and diarrhea are, however, all very common during pregnancy. In fact, it is estimated that that around 60% of all pregnant women experience at least one infection while pregnant.[65] The neurodevelopmental consequences of prenatal exposure to such common infections, is however less clear, and further scientific investigations are needed.

The biological mechanisms through which prenatal infections may cause neurodevelopmental impairment is not clearly understood. Some infections are transmitted directly from the mother to the fetus during pregnancy, as is the case with several of the TORCH infections.[66,67] Vertical transmission of virus from the mother to the fetus may have a direct adverse effect on brain development. However, in many of the common infections, vertical transmission is believed to be rare. An indirect pathway, involving maternal immune system activation is consequently receiving increased attention.[57,68] It is argued, that the fact that specific neurodevelopmental outcomes have been linked with host of different infections, suggests that factors common for infections, rather than the infection itself, may be the underlying etiologic factor for increasing disease risk.[57,69] Research efforts have primarily focused on immunological responses, such as cytokines and maternal fever. This thesis specifically aimed to examine the role of fever.

2.4 Prenatal fever exposure

When infection during pregnancy occurs, the maternal immune response is activated and fever may arise. It is hypothesized that cellular processes during fetal brain development, such as proliferation, migration, differentiation, myelination, and apoptosis may become disrupted, as a result of the elevated maternal body temperature.[67,70] This disruption may then in turn cause neurodevelopmental impairment in the child, which may unfold as the child grows. In humans, it is difficult to disentangle potential harmful effects of fever from the infection itself, given that fever more or less exclusively occur in the presence of infections. In animal models, however, hyperthermia can be experimentally induced, for instance by emerging the animal in hot water.

In animal models, the teratogenic effects of maternal hyperthermia are consequently well established.[71-73] Studies have implicated elevated body temperature, as a marker of maternal fever, in a range of adverse outcomes such as fetal growth restriction, malformations, fetal death, behavioral alterations, and impaired cognitive functioning.[73,74] While the animal studies suggest that many organs and structures are involved in hyperthermia-induced damage, the central nervous system seems to be particularly vulnerable to temperature effects during early development.[70] These findings have been consistent across a range of animal species,[72] and have furthermore demonstrated that the type of damage depends on the timing of the hyperthermic insult.[71-73]

In humans, hyperthermia most frequently occur as a result of fever, but may also be induced by elevated ambient temperatures, use of electric blankets, sauna or hot tubs, and vigorous exercise. Neurodevelopmental impairment has been demonstrated in epidemiological studies following in utero exposure to such external heat sources.[75-77] Given the body of evidence concerning the adverse effects of heat on the developing fetus from animal and as well as human studies, it was hypothesized that maternal fever in pregnancy could be a plausible mechanism through which prenatal infections affects fetal brain development. Findings of a systematic literature review of offspring health following prenatal fever exposure are presented in section 4.1.

2.5 Neurodevelopmental impairment following prenatal exposure to fever and infections

A majority of the literature on the neurodevelopmental consequences of common infections and fever during pregnancy have addressed conditions such as psychotic disorders,[78-86] autism spectrum disorders,[87-90] cerebral palsy,[91-93] neural tube defects,[77,94-102] and epilepsy.[103-105] Although the findings are not entirely consistent, a majority of the studies reported increased occurrence of these neurodevelopmental outcomes, following prenatal exposure to fever or common infections. While this evidence concerns profound teratogenic effects on brain development, there is also some evidence of less pervasive neurodevelopmental impairment, such as impaired motor skills[106] and more subtle psychological and behavioral outcomes.[107-109]

In this thesis, ADHD, academic performance and psychosis-like experiences were chosen to represent various measures of neurodevelopmental impairment in school-aged children. While these outcomes have not received much attention in the available literature, a few studies indicate that children who are prenatally exposed to fever, and various common infections such as genitourinary infections and respiratory infections, are at increased risk of having attention problems or ADHD,[107-113] low intelligence,[108,114-116] and psychosis-like symptoms.[117] Many of these studies are well-conducted, and are based on large samples with prospective data collection. However, given that the body of evidence is of

limited extent, it may be possible that the apparent consistency of findings is a result of publication bias. In addition, very few of these studies addressed how timing of prenatal exposure to fever and infection affected subsequent risk of neurodevelopmental impairment, and whether fever or the underlying infection was the contributing condition to increased disease risk. Assessment of prenatal exposures were also frequently based on hospital records or billing data, limiting the findings to more severe exposure episodes. Research specifically considering these gaps in the current knowledge is needed to improve our understanding of the neurodevelopmental risks associated with these common prenatal exposures and ultimately translating this into applicable knowledge for pregnant women experiencing fevers and infections.

Chapter 3

Materials and methods

3.1 Systematic review and meta-analyses

A literature review of epidemiological studies was conducted as an initial step, to summarize and analyze the range of associated health consequences in the offspring following exposure to maternal fever in pregnancy (**paper 1**). A systematic approach to identifying studies, assessing eligibility, extracting information from studies, assessing risk of bias, and synthesizing findings, was used, according to the Cochrane Handbook for Systematic Reviews of Interventions.[118]

3.1.1 Inclusion of studies

A two-stage search strategy was applied to identify all relevant studies for inclusion. First, we systematically searched bibliographic databases (PubMed, Web of Science, and the Cochrane Library) using the following search words: *fever*, *febrile*, *hyperthermia*, *pyrexia*, *pregnancy*, *pregnant women*, and *gestation*. Next, we used a snowballing technique, in which we pursued references of references, to find studies not identified in the database search. Studies were initially screened by title and abstract and subsequently through full-text evaluation. Cohort and case-control studies addressing health outcomes of prenatal fever exposure in humans were eligible for inclusion in the review. Studies were included only if they explicitly considered fever or a febrile illness, as a proxy for fever. We excluded studies published before 1990, studies considering only selected populations, and studies with inadequate description of study methods.

3.1.2 Synthesis of studies

To quantitatively combine results of studies addressing the same outcome, meta-analyses were performed when ≥4 were eligible for inclusion. Statistical heterogeneity was assessed using Cochrane's χ^2 statistic. We used a Mantel-Haenszel fixed effects model and a DerSimonian and Laird random effects model to estimate a pooled odds ratio (OR), for homogenous and heterogeneous studies, respectively. Health outcomes addressed by fewer studies were summarized in tables and synthesized qualitatively.

3.2 Study design

Next, three studies of the neurodevelopmental consequences following prenatal exposure to fever and infection were conducted (**papers 2-4**). The studies were based on the Danish National Birth Cohort, and the children were followed using registry- or questionnaire-based follow-up. All studies were based entirely on existing data sources, which were linked using maternal or child civil registration number. **Figure 2** provides a graphical illustration of the design of the three studies.
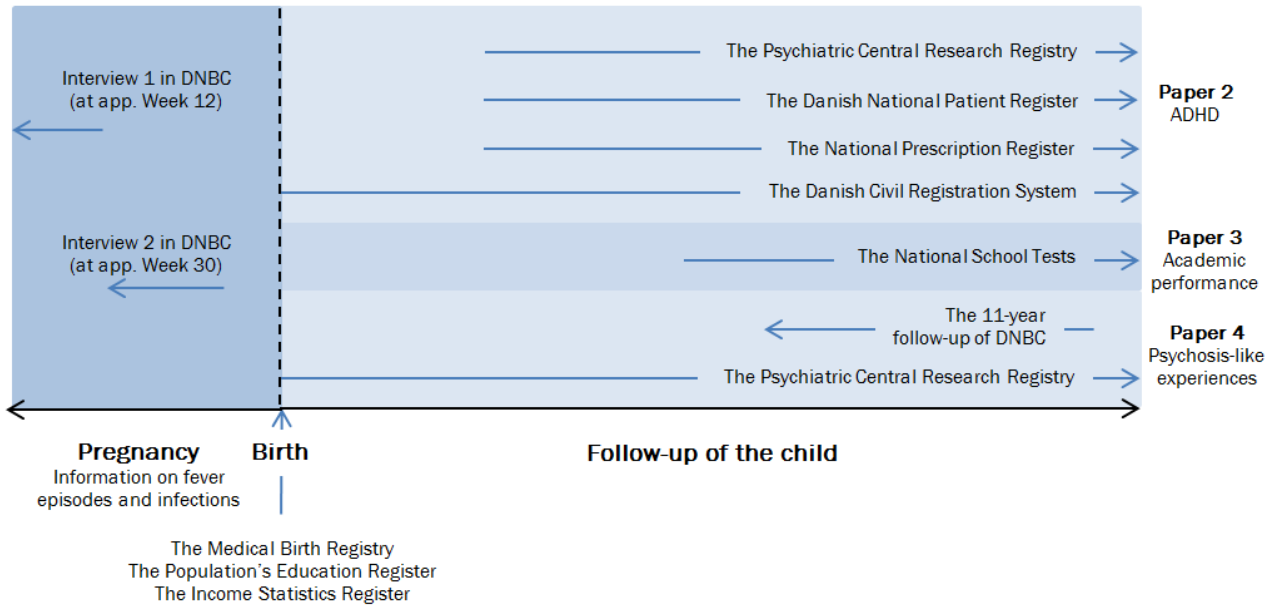
Figure 2: Overview of study designs.

3.3 Data sources

3.3.1 The Danish National Birth Cohort (DNBC)

The Danish National Birth Cohort is a nation-wide cohort study of pregnant women and their offspring.[119,120] Expecting mothers were invited to participate in the study during 1996-2002 by their general practitioner at the first antenatal visit, which usually takes place in pregnancy week 6-10. All general practitioners in Denmark were asked to take part in the recruitment of pregnant women, however not everyone did so. The actual number of women invited into the cohort is unknown, and an accurate estimation of the participation rate is consequently not possible. A participation rate of around 60% among invitees has however been suggested.[121] During pregnancy, two telephone interviews were scheduled to take place; one around pregnancy week 12 and another around pregnancy week 30. The women were asked about a range of lifestyle and health-related questions. In order to be eligible for inclusion the pregnant woman had to reside in Denmark, intend to carry the pregnancy to term, and have sufficient proficiency in Danish to complete the telephone interviews. Follow-up of the children has been conducted at 6 months of age, 18 months of age, 7 years, 11 years and the 18-year follow-up is currently

ongoing. In the studies presented in this thesis, data from the two pregnancy interviews, and the 11-year follow-up is used. Written informed consent was obtained from all cohort participants before entering the study. The use of data from the DNBC in this thesis has furthermore been approved by the DNBC steering committee (reference number 2012-30 and 2015-45) and the Danish Data Protection Agency (journal number: 2013-41-1431).

3.3.2 The Danish Registers

A range of different health and social registers were used in this thesis. By linkage using the civil registration number, we were able to follow each child in the registries from the day of birth and to extract information on potential important confounders. **Table 1** summarizes main characteristics of registers relevant to this thesis, and the information used in the three studies.

Table 1: Characteristics and use of Danish registries in the thesis.

Name of register	Coverage *(years extracted)*	Study unit	Extracted information
The Psychiatric Central Research Register[122]	All admissions to psychiatric hospitals and departments in Denmark. Since 1995 outpatient and emergency room contacts are included as well *(1977-2011)*	Hospital contacts (multiple records/person)	**Paper 2** Child diagnosis of ADHD, date of initial diagnosis. **Paper 4** Any child, maternal and paternal diagnosis of psychiatric disorders.
The Danish National Patient Register[123]	All admissions, outpatient and emergency room contacts to hospitals in Denmark. *(1997-2010)*	Hospital contacts (multiple records/person)	**Paper 2** Child diagnosis of ADHD, date of initial diagnosis.
The Danish National Prescription Registry[124]	All dispensed prescriptions of medication from all Danish pharmacies. *(1997- 2011)*	Redeemed prescriptions (multiple records/person)	**Paper 2** Redeemed prescription for ADHD medications, date of first redeemed prescription.
The Danish Medical Birth Registry[125]	All hospital and home births in Denmark. *(1997-2003)*	Birth (1 record/person)	**Papers 2-4** Date and year of birth, birth weight, gestational age at delivery, gender, paternal civil registration number, place of birth, maternal age, maternal smoking status.
The Population's Education Register[126]	All persons attending an education in Denmark. *(1981-2003)*	Educational level (multiple records/person)	**Papers 2-4** Highest level of completed education, highest level of ongoing education.
The Income Statistics Register[127]	All persons economically active in Denmark. Builds on smaller registers (Central Taxpayer's Register, and the Salary Information Register etc.) *(1996-2002)*	Yearly household income (multiple record/person)	**Papers 3-4** Household income in the calendar year prior to birth.
Danish Civil Registration System[128]	All persons alive and living in Denmark. Continuously updated information on vital status, and place of residence. *(1996-2010)*	Changes in vital status or place of residence (multiple records/person)	**Paper 2** Child vital status, emigration, date of events.

3.3.3 The Danish National Test Program

In 2010 the Danish National Test Program [De nationale tests] was introduced as a series of compulsory tests to measure academic performance in primary and lower secondary schools [Folkeskolen] across Denmark. We were granted access to the data through the National Agency for Quality and Supervision, Ministry for Children, Education and Gender Equality. Because the test program has only recently been introduced, the use for research purposes has been very limited.[129,130] Thus, the results presented in this thesis (**paper 3**), constitutes one of the first applications of these data within health research. The tests are mandatory for all public schools, and covers approximately 85% of all children living in Denmark. Language assessments are conducted in 2nd, 4th, 6th, and 8th grade, while math assessments are conducted in 3rd and 6th grade. In this thesis, all available assessments from children in the DNBC in the years 2010-2013 was extracted. **Figure 3** depicts the available assessments for children born in each year between 1997 and 2003 in a lexis diagram.
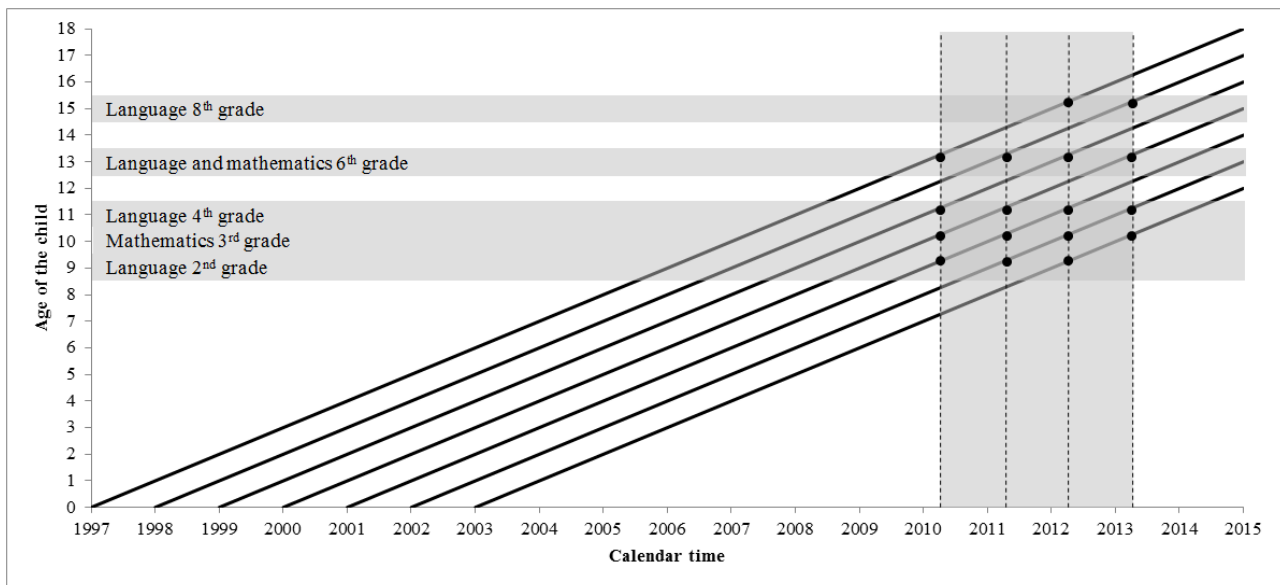
Figure 3: Graphical representation of assessments of academic performance available for children in the DNBC depending on their year of birth.

3.4 Study population

Children born in the DNBC were the underlying study population of all three studies. The final samples for analysis did however vary between the studies, mainly because the proportion of missing information on the three outcomes was very different, see **Figure 4**.
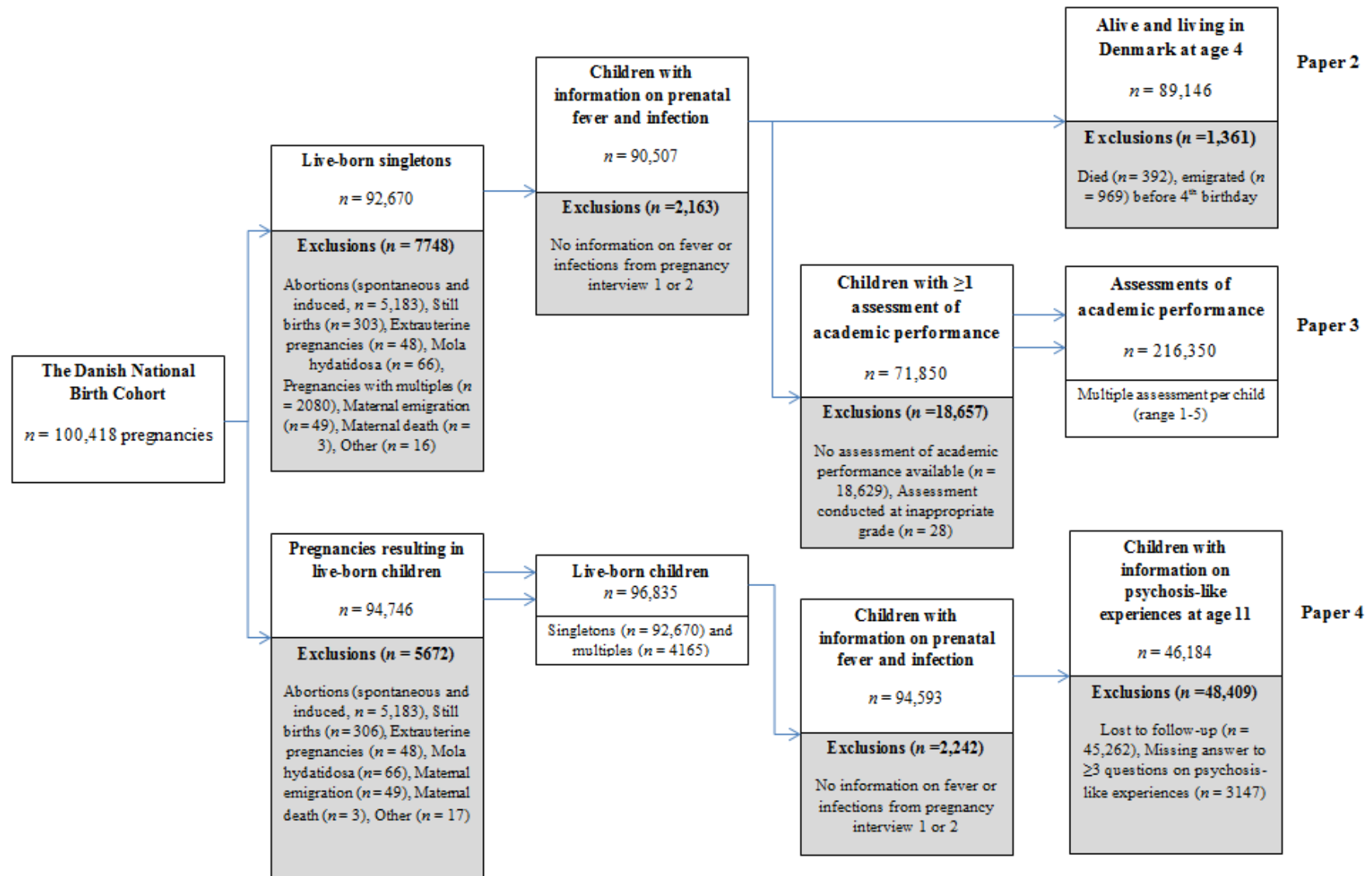
Figure 4: Flow-chart of study populations in this thesis.

3.5 Exposures

Maternal exposure to fever episodes and a range of common infections was assessed using either the first, second, or both pregnancy interviews. **Appendix 1** contains a full list of the questions used to address these exposures.

3.5.1 Fever

Maternal fever in pregnancy was measured in both pregnancy interviews. The woman was asked "In this pregnancy/since the last interview, have you had any episodes of fever?" and she was able to answer "yes", "no", "don't know", or "do not want to answer". A woman was considered exposed if she at any time in pregnancy reported having had an episode of fever. She was contrarily considered unexposed if she did not report any episodes of fever and answered no to the questions concerning fever in one or both interviews, see **Table 2**.

Table 2: Defining fever exposure at any time during pregnancy.

		Interview 1 – Fever				
		Yes	No	Don't know	Do not want to answer	Missing
Interview 2 Fever	Yes	*Exposed*				
	No		*Unexposed*			
	Don't know					
	Do not want to answer			*Exposure status missing*		
	Missing					

Up to ten episodes of fever were registered per interview. For each episode, information on accompanying symptoms, duration of fever (in days), maximum temperature during the fever episode (in °C) and timing of exposure (in gestational weeks) was recorded as well. Exposure episodes reported in the second interview were removed, if they occurred before the time of the first interview, in order to diminish double registrations. For each woman we also removed episodes that were registered with a gestational week occurring later than the time of her last interview, as this information was considered invalid.

Temperature

Fever intensity was defined according to maximum temperature (<39 °C, ≥39 °C, or unknown) among all fever episodes in each pregnancy in **papers 3-4**, and as a function of duration and temperature (low: 1 day ≥39 °C or 1-2 days <39 °C; medium: 2 days ≥39 °C or ≥3 days <39 °C; or high: ≥3 days ≥39 °C) in **paper 2**. If a woman was not able to recall the duration or the maximum temperature of the fever, the given episode was assigned a duration of 1 day and a maximum temperature <39 °C, as a conservative

estimate in **paper 2**. In **papers 3-4**, women reporting unknown maximum temperatures were considered as a separate group.

Timing

Timing of fever exposure was also handled differently in the three studies. In **papers 2-3**, ten four-week periods were defined (gestational weeks 1-4, 5-8, 9-12, …, 37-40), with binary variables indicating exposure status for each period (yes/no). Only women with complete information on all four weeks were included in each of the variables (i.e. the timing of her last interview exceeded the last gestational week in the period). In **paper 4**, the sample of children was substantially smaller, due to loss to follow-up. Thus, to ensure adequate numbers in these sub-groups we considered timing of exposure according to trimester (1st trimester: gestational week 1-12 and 2nd trimester: gestational week 13-28). Again, a woman was only included in the trimester-specific analyses if exposure information on the entire trimester of interest was available (i.e. the timing of her last interview exceeded the last gestational week in the trimester). Because only a very little and highly selected group of women had information up until gestational week 40, effects of third trimester exposure were not estimated.

3.5.2 Infections

Information on exposure to infections during pregnancy was primarily based on the second interview. The women were specifically questioned about a range of common infections and at which gestational week exposure had occurred. As for fevers, the women were able to report multiple episodes of each infection and the principles for defining any-time in pregnancy and timing-specific exposure to infections, were similar to those for fever. Information was available on genitourinary infections (cystitis, pyelonephritis, vaginal symptoms), persistent viral infections (orofacial herpes infection, genital herpes infection, condylomas), respiratory tract infection (prolonged cough), and diarrhea. Exposure to any infection was defined as the occurrence of any of these infections or if the mother replied "yes" to the question "In this pregnancy, have you had any other infections?". Given that an influenza diagnosis is rarely confirmed and self-diagnosis has poor predictive value,[131] women were not asked directly about their exposure to influenza. Instead, we a priori decided to define a fever-episode accompanied by headache and muscle or joint pain as a case of influenza-like illness. However, due to different definitions being used in the existing literature,[132] three other alternative case-definitions were examined as well (fever episodes with varying combinations of influenza symptoms), for instance with reference to definitions suggested by the World Health Organization and the Centers for Disease Control and Prevention.[133,134]

The exposures considered in each of the three studies varied somewhat, see **Table 3**. Persistent viral infections were only analyzed in paper 2, and not the subsequent two, because the primary infection did

not necessarily occur during pregnancy. Influenza-like illness was furthermore not considered in papers 2-3, because access to relevant data was not obtained until fall 2016.

Table 3: Overview of prenatal exposures examined in the thesis.

Prenatal exposure	Paper 2 ADHD	Paper 3 Academic performance	Paper 4 Psychosis-like experiences
Fever	x	x	x
Genitourinary infections	x	x	x
Respiratory tract infections	x	x	x
Diarrhea	x	x	
Persistent viral infections	x		
Any infection	x	x	
Influenza-like illness			x

In the interview conducted six months post-partum, the mother furthermore reported whether she had experienced fever or infections in the last part of pregnancy (i.e. after the second interview). These data were not used in any of the three studies, because it was expected that the accuracy of recall (e.g. timing of exposure) would vary substantially between in-pregnancy and after-pregnancy reporting. Additionally, given that children with neurodevelopmental impairment may differ from other children already in infancy (e.g. excessive crying), use of such information could potentially induce recall bias.

3.6 Outcomes

The three neurodevelopmental end-points assessed in this thesis were ascertained using a combination of register- and questionnaire-based data sources.

ADHD cases among children in the DNBC were identified using three nation-wide registers. Each child was followed from their 4th birthday and was defined as having ADHD if a diagnosis (ICD-10 DF90.0-DF90.9) was registered in the Psychiatric Central Research Register or the Danish National Patient Register or by redeemed prescription for central nervous system stimulant medications, in the Danish National Prescription Registry, see **Figure 5**. Most cases were identified in the Danish National Prescription Register, and approximately 52% of all cases were identified in at least two registers.
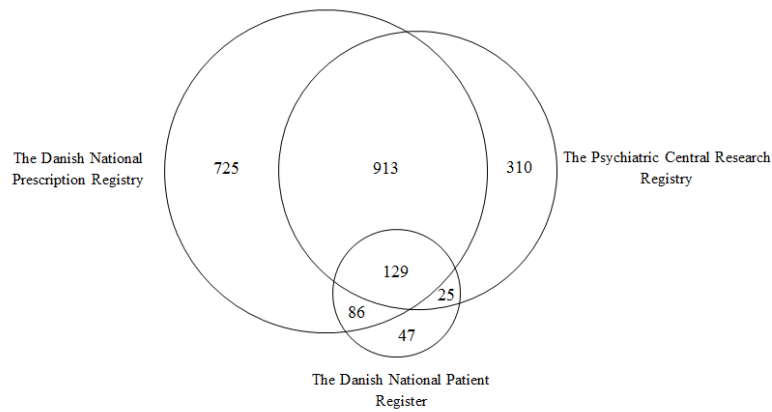
Figure 5: Identification of ADHD cases in Danish registers and their overlap.

Academic performance in the children was ascertained using language and math assessments from the Danish National Test Program. Results were Rasch scores,[135] and provided an adaptive measure of cognitive ability, called the estimated pupil ability (EPA). EPA was measured on a log-scale ranging from -7 to 7 with higher scores indicating higher ability. The EPA was provided for three profile areas for each language (language comprehension, decoding, and reading comprehension) and each math (numbers and algebra, geometry, and applied mathematics) assessment. In this thesis we used a summarized score for each assessment, representing the average EPA across the three profile areas. The median number of assessments per child was three, but as many as five assessments were available for some children.

Psychosis-like experiences were assessed by self-report in a web-based questionnaire completed by the children themselves at age 11. A seven-item Adolescent Psychotic-Like Screener, [136] partly based on the Diagnostic Interview Schedule for Children[137] was devised to the children, see **Table 4**. The children could answer "yes, definitely", "yes, maybe", and "no". Psychosis-like experiences were analyzed as a summarized variable according to the presence or absence of any definite symptoms (0, 1 or ≥2 definite symptoms).

Table 4: Questions concerning psychosis-like experiences in the 11-year follow-up of the Danish National Birth Cohort.

Item	Question
1	Some people believe that other people can read their thoughts. Have other people ever read your thoughts?
2	Have you been sent messages through the television or the radio, just for you alone?
3	Have you ever thought you were being followed or spied on?
4	Have you ever heard voices or sounds that other people couldn't hear?
5	Have you ever felt that you were under the control of some special power?
6	Have you ever seen things that other people could not see?
7	Have you ever felt that you have special powers?

For an overview of the neurodevelopmental endpoints included in this thesis, see **Table 5**.

Table 5: Overview of outcomes included in the thesis.

Outcome	Outcome definition	Age of child at assessment	Non-cases/ Cases	Data
ADHD (paper 2)	Diagnosis of ADHD or redeemed prescription of ADHD medication *Date of first appearance in registers*	10.6 years[a]	86,911/2235	The Psychiatric Central Research Register and the Danish National Patient Register *ICD-10 Diagnoses: DF90.0-DF90.9* The Danish National Prescription Registry *ATC codes: N06BA02, N06BA04, N06BA09*
Academic Performance (paper 3)	Summarized EPA for each assessment in the Danish National Test Program *Numerical, log-scale*	8-15 years	-	The Danish National Test Program *Language assessments: 2nd, 4th, 6th, 8th grade* *Math assessments in 3rd, 6th grade*
Psychosis-like experiences (paper 4)	Number of definite psychosis-like experiences *Ordinal (0, 1, or ≥2)*	11 years	40,898/3434 /1852	11-year follow-up of DNBC *7-item Psychotic-Like Screener*

Abbreviations: ADHD: Attention Deficit/Hyperactivity Disorder; ATC: Anatomical Therapeutic Chemical Classification Code; DNBC: Danish National Birth Cohort; EPA: Estimated Pupil Ability; ICD-10: International Statistical Classification of Diseases and Related Health Problems, 10th revision.
[a]Mean time of follow-up

3.7 Covariates

Selection of covariates in the three studies was generally based on a priori discussions of potential confounders of the exposure-outcome relationship. Existing evidence was used to guide this selection. In the study of ADHD, the principles underlying directed acyclic graphs (DAGs),[138,139] was furthermore applied to visualize assumptions of causal paths between exposures, outcome and other variables of interest (see **paper 2**). Although our model was not necessarily entirely exhaustive, the process of constructing the diagram, facilitated a better understanding of potential confounding mechanisms and under which circumstances bias might occur.

3.8 Statistical analyses

All analyses presented in this thesis were conducted via remote access to the Research Machines in Statistics Denmark, using Stata (StataCorp, College Station,Texas).

In all three studies, characteristics of the study populations were presented as an initial descriptive analysis. Categorical and numerical variables were presented in tables by their relative frequencies, and means with standard deviations, respectively. For non-normal numerical variables the median and range was presented instead. Comparisons between groups were conducted using Pearson's χ^2-test.

In the main analyses of each study, various regression analyses were used to analyze the relationship between prenatal exposure to fever and infections and the outcome of interest, see **Table 6**.

Table 6: Main statistical analyses applied in the thesis.

Study	Model (*estimate*)	Interpretation of estimate
ADHD (Paper 2)	Cox regression (*Hazard ratio, HR*)	The rate of ADHD among children prenatally exposed to fever or infection relative to children that are unexposed.
Academic performance (Paper 3)	Multi-level linear regression (*exp(β)=odds ratio, OR*)	The odds of answering any given question in the test correctly among children prenatally exposed to fever or infection relative to children that are unexposed.
Psychosis-like experiences (Paper 4)	Multinomial logistic regression (*Relative risk ratio, RRR*)	The risk of having experienced one [≥two] rather than none psychosis-like symptom[s], among children prenatally exposed to fever or infection relative to children that are unexposed.

The analyses in all studies aimed to estimate associations with the outcome in relation to 1) exposure to prenatal fevers and infections at any time during pregnancy, 2) exposure to prenatal fevers and infections during specific times of pregnancy, and 3) exposure to different intensities of fever (temperature analyses). All measures of association were estimated using unadjusted and adjusted models. However, due to restrictions in space, not all crude analyses were presented in **papers 2-4**. In order to distinguish between the effects of fever and infection, we furthermore adjusted all analyses concerning fever for the effect of infections and vice versa. Sensitivity analyses did, however, reveal that excluding adjustment for infections/fever essentially left the findings unchanged.

3.8.1 Handling loss to follow-up

Missing information on the outcome was a concern in **papers 3 and 4**. For academic performance, assessments were missing for approximately 20% of all children, while around 50% of the children were lacking information on psychosis-like experiences at age 11. In complete-case analysis, it is implicitly assumed that the data is missing completely at random. However, in the DNBC loss to follow-up has been shown to depend on a host of variables, for instance parental socioeconomic background,[140] and similar findings have been noted for participation in the National Test Program.[130,141] In both papers, we evaluated the extent of potential bias, by comparing the estimates from standard regression models, with those of models with applied inverse probability weights.[142] Using a logistic regression model, we calculated a weight for each child proportional to the inverse probability of not being missing, given the specified covariates. In essence, children that were not missing, but with a high chance according to the model of being so, was given a higher weight. In the weighted analyses these children were then not only representing themselves, but also the other children like them, that were not observed. The models aimed

to include all relevant variables related to missingness and it was then assumed that the information on the outcomes were missing at random, given the variables in the model.

3.9 Supplementary analyses

Misclassification of exposure and outcomes constitutes important potential sources of bias. In this thesis, some misclassification is expected, for instance in the analyses considering prenatal fever exposure at any time during pregnancy. For approximately 90% of the women included in the DNBC, we had exposure information until gestational week 28 (i.e. end of second trimester). However, at gestational week 36, this number had dropped to around 13% (because the 2nd interview was scheduled to be at gestational week 30). Thus, it seems plausible that for a non-trivial part of the women, their exposure status for any time in pregnancy exposure, might have been misclassified, due to the missing information in the last part of pregnancy. As a supplementary analyses, a multidimensional bias analysis[143] was conducted, for various combinations of sensitivity for prenatal fever exposure classification. As an example, numbers from **paper 2** on ADHD was used. A graphical illustration of misclassification for any-time in pregnancy exposure is shown in **Figure 6**.
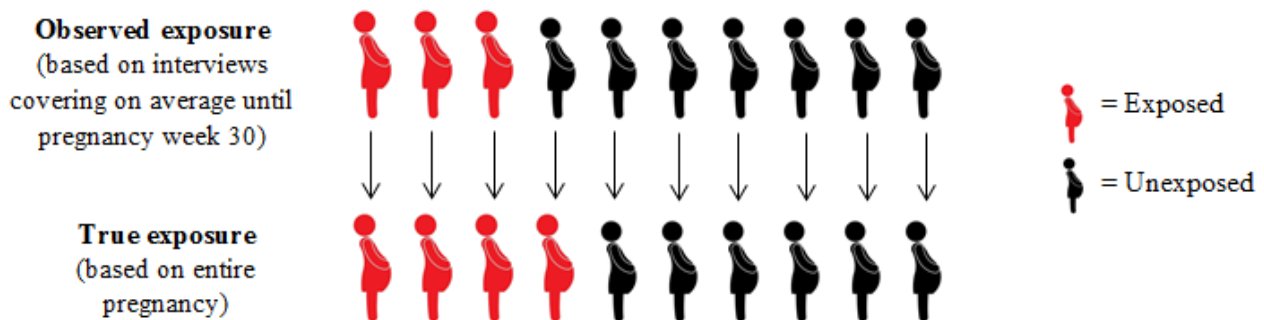
Figure 6: Misclassification of any-time in pregnancy exposure. The upper row represents classification of exposure at any time of pregnancy based on information collected in the two pregnancy interviews, while the bottom row represents the same women's true exposure status, if information had been available for the entire pregnancy. The figure illustrates that woman number four, is incorrectly classified as unexposed in the top row (i.e. a false negative). This misclassification occur, because she was not exposed prior to her last interview, but after. Contrarily, there are no false positives, because women classified as exposed based on observed data (i.e. exposed in first part of pregnancy), will be truly exposed irrespective of their exposure in the later part of pregnancy. A perfect specificity of 1.0. was therefore assumed (specificity=true negatives/true negatives+false positives). In this bias analysis perfect recall is furthermore assumed (i.e. reports of exposure corresponds to actual exposure).

Although we did not expect misclassification to differ substantially according to outcome status, scenarios assuming both non-differential and differential misclassification were evaluated. ORs corrected for misclassification were estimated using cell counts adjusted for different sensitivities, see **Table 7**.

Table 7: Exposure misclassification. Equations for calculating expected true data given the observed data with exposure misclassification.[143]

| | Observed | | Expected | |
	E+	E-	E+	E-
D+	a	b	$A = [a - D_+ \times (1 - SP_{D_+})]/[SE_{D_+} - (1 - SP_{D_+})]$	$B = D_+ - A$
D-	c	d	$C = [c - D_- \times (1 - SP_{D_-})]/[SE_{D_-} - (1 - SP_{D_-})]$	$D = D_- - C$
Total	$a + c$	$b + d$	$A+C$	$B+D$

[a]Exposure status: E+/E-, Disease status: D+/D-, Sensitivity: SE, Specificity: SP

Furthermore, outcome misclassification was also a potential concern, particularly in **paper 4**. Psychosis-like experiences were addressed by a screening questionnaire, but it has been suggested that endorsement in such questionnaires does not accurately predict actual psychosis-like symptoms, when followed up by probing questions, for instance in a clinical examination.[144] Thus, to address the impact of potential misclassification of psychosis-like experiences, a probabilistic bias analysis was conducted according to recently developed methods.[145,146] In brief, because the true sensitivities and specificities are rarely known with certainty, probabilistic bias analyses allows for an inclusion of likely ranges of values. Thus, in this supplementary analysis, trapezoidal probability distributions of likely values for sensitivity and specificity of outcome classification were defined (Sensitivity; minimum: 0.50, mode 1: 0.70, mode 2: 0.80, maximum: 1.0 and specificity; minimum: 0.95, Mode 1: 0.97, Mode 2: 0.98, Maximum 1.0). Choice of ranges for bias parameters (i.e. sensitivity and specificity) were guided by a recent validation study.[136] Values for sensitivity and specificity were then randomly sampled from these distributions, and applied to estimate the expected true cell counts and corrected ORs as described in **Table 8**.

Table 8: Outcome misclassification. Equations for calculating expected true data given the observed data with disease misclassification.[143]

| | Observed | | Expected | |
	E+	E-	E+	E-
D+	a	b	$A = \dfrac{[a - E_+ \times (1 - SP_{E_+})]}{[SE_{E_+} - (1 - SP_{E_+})]}$	$B = \dfrac{[b - E_- \times (1 - SP_{E_-})]}{[SE_{E_-} - (1 - SP_{E_-})]}$
D-	c	d	$C = E_+ - A$	$D = E - B$
Total	$a + c$	$b + d$	$A+C$	$B+D$

[a]Exposure status: E+/E-, Disease status: D+/D-, Sensitivity: SE, Specificity: SP

A total of 1000 simulations were conducted assuming non-differential misclassification, and the median OR and the corresponding 95% simulation interval was presented for the conventional and corrected analyses, respectively. This bias analysis consequently allowed for a quantification of the bias as well as

an estimation of the uncertainty due to random error (conventional analysis), due to systematic error (corrected analyses), as well as due to the total error (combined random and systematic error, for technical details see[145]). Given that these methods are developed for misclassification of binary variables, the impact of outcome misclassification was illustrated for auditory hallucinations, which was the most frequently reported psychosis-like experience (approximately 6% of the children). The results of the bias analyses are presented at the end of chapter 4.

Chapter 4

Results

4.1 Review of health consequences of prenatal fever exposure (paper 1)

A total of 46 studies were included in the systematic literature review. Substantial evidence was found to support the contention that maternal fever during pregnancy may negatively affect offspring health. The strongest evidence, from three meta-analyses, indicated that first trimester exposure was associated with neural tube defects (OR= 2.90, 95% CI: 2.22-3.79), heart defects, (OR=1.54, 95% CI: 1.37-1.74), and oral clefts, (OR=1.94, 1.35-2.79). The harmful effects seemed to cover both short- and longer-term health outcomes, although evidence on long-term effects was more sparse. Studies concerning neurodevelopmental impairment did suggest a potential adverse effect of prenatal fever exposure for autism,[87,89,90] cerebral palsy,[92,93] academic performance[108] and psychosis,[79] but not for epilepsy[103] or schizophrenia.[80] However, the evidence on these outcomes was insufficient to judge any association with certainty. This constituted the basis for the subsequent three studies, presented in this thesis.

4.2 ADHD following prenatal exposure to fever and infections (paper 2)

The first study aimed at examining ADHD occurrence among children exposed and unexposed to maternal fevers and infections during pregnancy. Overall, prenatal exposure to fever and a range of common infections was generally not, or only slightly associated with ADHD occurrence in the child. For the analyses of any-time in pregnancy exposure, only genitourinary infections was associated with a slightly elevated hazard rate (HR: 1.14, 95% CI: 1.03-1.25). The timing specific analyses revealed that this effect was primarily explained by a more substantial increase in the latter part of pregnancy (gestational week 29-32; HR: 1.22, 95% CI: 0.98-1.53, and week 33-36; HR: 1.60, 95 % CI: 1.13-2.26), which persisted even when analyses were restricted to term births (i.e. gestational week ≥37). For prenatal fever exposure, increasing intensity of the episode was not associated with increasing rates of ADHD, as we would have expected if a causal association existed. However, the timing analyses revealed that fever during gestational week 9-12 was associated with increased ADHD occurrence (HR: 1.33, 95% CI: 1.12-1.58), although this result was considered to be due to multiple testing. Thus, with the exception of genitourinary infections, no overall association was generally demonstrated between these prenatal exposures and subsequent risk of ADHD. The analyses do however indicate that prenatal exposure during specific time periods of pregnancy might be important when analyzing ADHD occurrence.

4.3 Academic performance following prenatal exposure to fever and infections (paper 3)

In the next study, academic performance among children within the DNBC was examined according to prenatal fever and infection exposure. A total of 216,350 assessments of academic performance were available for 71,850 children born to 67,528 mothers. The analyses revealed that neither prenatal exposure to fever nor any of the examined infections was associated with academic performance in

childhood and early adolescence. Odds ratios ranged from 0.99 to 1.01 and 95% confidence limits did not exceed ±0.03. These results were consistent for different types of assessments (language vs. math), for specific profile areas, and for various time windows of pregnancy. Furthermore, in a sensitivity analysis no differences in academic performance was observed among exposure discordant siblings, indicating that unmeasured confounding (e.g. by maternal intelligence) did not affect the findings. Finally, results from analyses using inverse probability weights were essentially identical to those of the unweighted analyses, suggesting that missing data on academic performance did not bias the results. In summary, all analyses consistently showed that academic performance in childhood and early adolescence was unrelated to maternal exposure to fever and common infections during pregnancy.

4.4 Psychosis-like experiences following prenatal exposure to fever and infections (paper 4)

In the final study, the association between maternal fevers and common infections in pregnancy and subsequent psychosis-like experiences in the children was examined. Eleven percent of the 46,184 children participating in the 11-year follow-up reported one or more definite psychosis-like symptoms. Maternal fever, genitourinary infections and respiratory infections during pregnancy were generally not or only weakly and inconsistently associated with psychosis-like experiences in the child. The association with influenza-like illness was very sensitive towards how influenza-like illness was defined, but when using our a priori definition (fever with accompanying headache and muscle or joint pain), influenza-like illness did seem to increase the occurrence of psychosis-like symptoms in the child. The association increased with increasing number of psychosis-like symptoms (RRR of one definite psychosis-like symptom= 1.16, 95% CI: 0.94-1.44, and RRR for ≥two definite symptoms= 1.38, 95% CI: 1.06-2.79), for and for increasing temperature of the fever episode. However, when broader definitions were applied (e.g. fever and one respiratory symptom), such associations disappeared. In the analyses of timing of exposure, no substantial evidence was found to support trimester-specific effects. Adjustment for selective attrition by use of inverse probability weights only affected the associations with fever, and essentially left all other associations unchanged.

4.5 Supplementary analyses

4.5.1 Representativeness of births in the DNBC

Although the DNBC was intended as a population-based birth cohort, selection processes inevitably affects participation. To examine the representativeness of the cohort, births within the DNBC were compared to all births in Denmark during the same time period (1997-2003), see **Table 9**. As expected, women participating in the DNBC were more likely to be primiparous, to be of higher socioeconomic status (higher education and income), and to be in better health (less likely to smoke, and give birth

premature, and higher mean birth weight in babies) compared to the entire population of women giving birth between 1997 and 2003 in Denmark. This pattern most likely reflects that resources in terms of time, knowledge, money, and health, were somewhat higher among DNBC mothers. The study populations underlying the findings presented in sections 4.2-4.4, were consequently not entirely representative for all children living in Denmark.

Table 9: Characteristics of births in the Danish National Birth Cohort and all births in Denmark between 1997 and 2003.

Characteristics	Danish National Birth Cohort $n = 94,782^a$	All births 1997-2003 $n = 463,310$
Maternal age at birth		
≤24 years	9.6	14.7
25-29 years	38.3	36.0
30-34 years	37.1	34.2
≥ 35 years	14.9	15.1
Gestational age at delivery		
<37 weeks	5.5	6.6
37-41 weeks	85.5	84.7
>42 weeks	8.7	8.2
Missing	0.3	0.6
Parity		
Primiparous	47.4	43.4
Biparous	36.5	36.9
Multiparous	16.1	19.4
Missing	0.0	0.0
Birth weight in grams, Mean (SD)	3557 (594)	3493 (617)
Maternal education at birth		
Primary education	11.5	19.7
Secondary education	42.6	42.7
Higher education	45.6	35.3
Missing	0.6	2.2
Household income in year prior to birth		
Lowest quintile	16.7	19.4
2nd quintile	16.3	19.4
Medium	20.3	19.4
4th quintile	22.5	19.4
Highest quintile	23.6	19.4
Missing	0.6	3.1
Maternal smoking in pregnancy		
Nonsmokers	79.5	74.3
Former smokers	2.0	1.8
Light smokers (1-9 cigarettes/day)	10.5	12.7
Heavy smokers (>10 cigarettes/day)	4.1	6.0
Smokers, unknown amount	0.4	0.5
Missing	3.5	4.7

[a] DNBC births registered in the Medical Birth Registry. The number includes live- and stillbirths and is consequently not identical to tables presented in papers 2-4, which only includes live-born children.

4.5.2 Exposure and outcome misclassification

Scenarios assuming different levels of misclassification of any-time in pregnancy exposure status for fever among children with and without ADHD are presented in **Table 10**. Grey cells represent scenarios assuming non-differential misclassification. Corrected ORs below the grey cells illustrate varying

scenarios with differential misclassification, where children with ADHD have higher levels of misclassification (i.e. lower sensitivity) than children without ADHD. Contrarily, corrected ORs above the grey cells represent differential misclassification where children with ADHD have lower levels of misclassification compared to children without ADHD. The table shows that non-differential misclassification only marginally underestimate the OR, even for very low sensitivities. Differential misclassification does, however, induce bias, with the direction depending on whether misclassification was greatest among children with or without ADHD. If misclassification was generally higher among children *with* ADHD, then the results of the conventional analyses underestimated the association. Contrarily, if misclassification was higher among children *without* ADHD, then the conventional analysis would overestimate the association. Given that the degree of misclassification would depend on when the last interview during pregnancy was conducted (i.e. the later the smaller risk of misclassification), the average week of the last interview was compared among children with and without ADHD. Mothers of children without ADHD was, on average, interviewed slightly later (gestational week 31.0) than mothers of children with ADHD (gestational week 30.8), p=0.026. However, although this difference reached statistical significance, it only corresponds to a difference of approximately 1-2 days. Thus, it seems reasonable to assume that the misclassification was non-differential, and that the results were not biased in any substantial manner by this issue.

Table 10: Multidimensional bias analysis of misclassification of fever at any time during pregnancy among children with and without ADHD.[a]

		Sensitivity of fever exposure among children without ADHD (D.)					
		1.0	0.90	0.80	0.70	0.6	0.5
		Corrected ORs[b]					
Sensitivity of fever exposure among children with ADHD (D+).	1.0	1.10	0.95	0.80	-	-	-
	0.90	1.29	1.11	0.93	0.75	-	-
	0.80	1.54	1.33	1.12	0.90	0.69	-
	0.70	-	1.66	1.39	1.13	0.86	0.60
	0.60	-	-	1.85	1.50	1.15	0.79
	0.50	-	-	-	2.23	1.71	1.18

[a] Analyses are based on the following observed cell counts in Table 7: a = 659, b = 1571, c = 23,872, d = 62,855 (conventional OR= 1.10, 95% CI: 1.01-1.21).
[b] Assumed specificity of 1.0

Probabilistic bias analysis was then used to examine potential misclassification of psychosis-like experiences (**Table 11**). The table is based on an example for auditory hallucinations and prenatal fever exposure, but could have been done for other exposures and other psychosis-like experiences as well. It was assumed that misclassification was non-differential, given that it was deemed unlikely that the children's answers depended on maternal exposure status. The analyses indicated that the effects of

prenatal fever seemed to be somewhat underestimated in the conventional analysis (OR=1.15, 95 CI: 1.06-1.25) compared to the analysis corrected for misclassification (corrected OR=1.27, 95% simulation interval: 1.17-1.62), given the specified ranges of likely values for sensitivity and specificity. However, when the analyses accounted for both random error (estimated by the conventional 95% CI) and systematic error (estimated by the 95% simulation interval), the combined uncertainty interval was somewhat broader, suggesting that the conventional analysis overestimated the precision of the results.

Table 11: Probabilistic bias analysis of misclassification of psychosis-like experiences (auditory hallucinations) among children prenatally exposed and unexposed to fever.[a]

	OR 2.5 percentile	OR median estimate	OR 97.5 percentile	Percentage change in interval width (97.5 percentile/2.5 percentile) compared with conventional
Conventional analysis Random error only	1.06	1.15	1.25	0
Corrected analysis[b]				
Systematic error only	1.17	1.27	1.62	17.4
Systematic and random error	1.13	1.27	1.64	23.1

[a]Analyses are based on the following observed cell counts in Table 8: a = 826, b = 1949, c = 11,649, d = 31,677

Chapter 5

Discussion

5.1 Summary of main findings

In the studies presented in this thesis, we did not find evidence that fever and common infections in pregnancy contribute substantially to longer-term neurodevelopmental impairment, assessed by the occurrence of ADHD, academic performance and psychosis-like symptoms. These findings were based on three studies conducted within the DNBC, where detailed information about prenatal exposure to fever and a range of different infections were collected prior to birth, and where children were followed in registers or questionnaire-based follow-up. Although the analyses suggested that these prenatal exposures *in general* were not or only weakly associated with neurodevelopmental impairment, a few exceptions are worth mentioning. For instance, prenatal fever exposure in early pregnancy was strongly associated with subsequent risk of neural tube defects in the systematic literature review. Genitourinary infections in late pregnancy was also moderately associated with ADHD occurrence in the child, and finally influenza-like illness during pregnancy, defined as episodes of fever with headache and muscle or joint pain, seemed to be associated with a somewhat increased risk of psychosis-like symptoms in the child. The overall findings are discussed in further details in the following sections.

5.2 General discussion and consistency with existing literature

In the systematic review (**paper 1**), substantial evidence was found to support a detrimental effect of fever during pregnancy on fetal brain development. The review was not limited to neurodevelopmental outcomes, although this discussion will only address findings in that context. The strongest and most consistent finding was the association between first-trimester fever exposure and neural tube defects. Although the nine studies included in the meta-analysis were heterogeneous, all ORs and corresponding 95% CIs were above one. These findings added to the results of a previously published review[94] that aside from fever in pregnancy linked other sources of prenatal heat exposure (e.g. sauna, hot blankets etc.) to neural tubes defects. Neural tube defects do however differ substantially from the other neurodevelopmental outcomes of interest in this thesis, given that it is a structural defect. Although there might be abnormal structural features in the brains of children with ADHD, impaired academic performance, and psychosis-like experiences, these deficits are primarily seen as functional defects of the brain.

In **paper 2,** ADHD occurrence in the child was generally not associated with overall maternal exposure to fever and a range of common infections during pregnancy, including prolonged cough, diarrhea, and persistent viral infections. While no other studies examining ADHD in relation to prenatal exposure to diarrhea and persistent viral infections were identified, a few studies reported contrasting findings for respiratory infections and fever. For instance, in a recently published longitudinal study of 534 mother-child pairs, upper respiratory infections during pregnancy were associated with increased attentional problems reported by mothers and teachers.[109] Similarly, in a study of 893 children conducted within the

Helsinki Longitudinal Project, second trimester exposure to fever was associated with impaired executive functions,[108] which are frequently reported in children with ADHD.[147] While such prenatal exposures may consequently increase the risk of non-clinical ADHD-like behaviors, our study suggests that these findings do not extend to clinically recognized cases of ADHD. These studies were also limited by much smaller samples ($n<1000$), and in some instances by retrospective collection of exposure information,[109,113] suggesting that findings may have been more prone to bias. Maternal report of genitourinary infection in pregnancy, was the only exposure examined in our study, that was associated with a slightly increased rate of ADHD occurrence in the child. The timing analyses suggested that while rates of ADHD did not seem to be affected by exposure in early and mid-pregnancy, exposure in third trimester was associated with a more substantial increase. These findings persisted even when analyses were restricted to term births, suggesting that these effects were not explained by prematurity of the child. These findings were consistent with two large register-based studies,[110,111] reporting an approximately 30% increased risk of ADHD among children prenatally exposed to genitourinary infections. The somewhat stronger associations reported in those studies (for any-time in pregnancy exposure), possibly reflects differences in how genitourinary infections were assessed (medical records and billing data versus self-reported data in our study). While these studies did not address the timing of exposure episodes, other authors have hypothesized that the last part of pregnancy may constitute a period of increased vulnerability towards insults from maternal infections in relation to offspring risk of ADHD.[148]

In the second study, our findings indicated that academic performance in the child was unaffected by prenatal exposure to fevers and any of the common infections examined (**paper 3**). Due to the availability of a large number of assessments (>200.000), we were able to estimate associations with a high precision and exclude that even very subtle differences in academic performance existed. While a few smaller studies have reported similar findings,[109,149] most of the existing literature suggests that cognitive ability may be adversely affected by such prenatal exposures.[108,114-116] In two large Scandinavian studies on young males, lower intelligence at conscription were reported for prenatal exposure to infections.[114,115] The magnitude of the effect was estimated to be a reduction of 1 point on a standard IQ scale in a Danish study,[114] and between 3 and 7 points in a Norwegian study.[115] In the study from Denmark, they only considered severe infections that required hospitalization during pregnancy. It is consequently possible that these findings do not generalize to the more common and mild infections considered in our study. The Norwegian study, on the other hand, was based on an ecological design, where aggregate data on prenatal influenza exposure and intelligence was used. Such studies are prone to ecological bias, because observed associations on aggregate level data (in this instance country-level), may not necessarily reflect a biological effect at the individual level.[150] In addition, in a recently published study on a sub-cohort of 1,491 children within the DNBC, maternal fever in pregnancy was associated with a reduction of 2.7 points on verbal IQ and 4.3 points on performance IQ at five years of age.[116] These findings may suggest

that prenatal exposure to fever and infections lead to a slight reduction in intelligence, however on the basis of our findings it seems that this reduction may not necessarily translate into impaired academic performance in school.

The hypothesis that prenatal infections and fever may be associated with psychosis-like experiences in the child was based on the growing evidence supporting the role of prenatal infections in the development of psychotic disorders, such as schizophrenia.[67,78,81,83] Given that psychosis-like experiences are considered as sub-clinical expressions of psychosis, it was hypothesized that prenatal infections and fevers might constitute common underlying risk factors. Only one study addressing the effect of prenatal infections on subsequent psychosis-like experiences in the child was identified.[117] In this study of 5379 children from the Avon Longitudinal Study of Parents and their Children (ALSPAC), the authors reported a 1.44 fold increased risk of non-clinical psychotic symptoms in children at age 12, if they were prenatally exposed to any infection (95% CI: 1.11-1.86). In the final study of this thesis (**paper 4**), we were not able to replicate these findings, but found that psychosis-like experiences at age 11 were not or only weakly associated with such prenatal exposures. Influenza in pregnancy has been widely examined in relation to subsequent psychopathology in the offspring, since Mednick published his study in the 1980s.[8] Our findings strongly depended on how influenza-like illness was defined, but using our a priori case-definition of influenza-like illness yielded estimates in support of a potential harmful effect of prenatal exposure. Using other definitions did, however, reduce or entirely remove those associations. Whether these findings were due to broader definitions being less specific to influenza, or more narrow definitions being more selective towards severe infections was unclear.

5.3 Thoughts on causality

The overarching question then remains whether the (few) associations observed were in fact causal. As causality cannot be proven, we rely on causal inference as the process of scientific reasoning and drawing conclusions about the causal nature of associations.[151,152] Various issues relating to the plausibility that fever and infections during pregnancy may be causally related to impaired neurodevelopment in the child is discussed in the following sections.

Timing-specific effects

It has been proposed that vulnerability to infection-mediated disturbances in fetal brain development and associated neurodevelopmental outcomes varies across different gestational periods.[153,154] For instance, it is well-established that the neural tube is normally fully formed by the end of gestational week 6,[155] and that exposure extending past this point of pregnancy would be unrelated to neural tube formation.[156] For the same reason, was assessment of fever in all studies, included in the meta-analysis in **paper 1**, limited

to the first few months of pregnancy. The specificity of the observed association to this particular period of pregnancy supported the possibility that it might be causal.

The current understanding of how specific periods of prenatal development is related to the other outcomes of interest in this thesis, is however far less clear. In most of the existing literature, timing-specific findings have been absent, with two notable exceptions suggesting that mid-pregnancy constituted the most vulnerable period for a range of psychological and educational outcomes.[108,109] Similarly, initially the second trimester was also suggested as the period of pregnancy associated with the greatest risk, in the association between schizophrenia and prenatal influenza exposure,[67] but many studies have since then failed to replicate these findings.[78,81] Other authors argue, that the neurodevelopmental impact of prenatal infections is greatest in early pregnancy, given that such infections not only interfere with neurodevelopmental processes at the time of the infection, but may also predispose the developing nervous system to additional failures in subsequent development.[154] Given the lack of consistency in the literature, we did not specify any a priori hypotheses in this thesis, concerning specific periods of increased vulnerability. While the risk of psychosis-like symptoms did not vary substantially for exposures across trimesters, some evidence of a timing-specific effect was found for the association between genitourinary infections and ADHD, which seemed to be limited to third-trimester exposure. While these findings were consistent with hypotheses presented by Strickland,[148] they should be interpreted cautiously. The quality of the data from the DNBC in the end of pregnancy, was limited by the fact that the final pregnancy interview was scheduled to take place at pregnancy week 30. The women with information on later pregnancy was consequently highly selected, for instance by the child's gestational age at birth. Whether the final trimester of pregnancy actually constitutes a critical developmental period for ADHD, should be addressed in future research.

Distinguishing between the effects of fever and the underlying infection
One of the main challenges to causal inference was to distinguish between the effects of elevated maternal body temperature from the underlying infection itself, because fevers and infections frequently co-occur. Intuitively, fever would be a plausible etiologic agent of increased disease risk if 1) higher temperatures during fever episodes shows stronger associations with neurodevelopmental outcomes and 2) associations are reduced or even eliminated when antipyretic medication is used to lower the temperature. However, the interpretation of such analyses is complicated by the fact that increasing temperatures of a fever episode correlates with the severity of the underlying infection, and also with potential treatment (e.g. antibiotics or antipyretics). Similarly, the indication for taking antipyretics would depend on severity of the fever and the underlying disease. Whether fever intensity merely acts as a proxy for infection severity or medication exposure is consequently difficult establish.

Dose-response relationship with temperature has only been examined in a few prior studies on neurodevelopmental outcomes. All of these studies were based on the children within the DNBC, and considered autism and associated spectrum disorders,[87] epilepsy,[103] and motor performance.[106] These studies were not able to demonstrate any differences in the strengths of the associations when high- and low-grade fevers were considered separately. For instance, in the study by Holst and colleagues, maternal fever was associated with developmental coordination disorder in the child at age seven (OR= 1.29, 95% CI: 1.12-1.49), but this association was not stronger for fevers with temperatures ≥39°C (OR= 1.25, 95% CI: 0.97-1.63) than fevers <39°C (OR= 1.20, 95% CI: 0.93-1.53).[106] These findings indicate that elevated maternal body temperature might not be the underlying etiologic agent of increasing disease risk, but rather associated or underlying causes of the fever. In the studies conducted as part of this thesis, increasing temperatures of the prenatal fever episode was also not associated with ADHD, academic performance or psychosis-like experiences (**papers 2-4**). The overall lack of a dose-response relationship in the literature and in the results presented as part of this thesis, suggests that fever may not constitute a risk by it-self for these neurodevelopmental outcomes. However, it should be noted that if there were any error related to measurement or reporting of temperature in the DNBC, such bias would have affected findings in all of these studies.

Somewhat contrasting these findings, is the literature examining the effect of treatment with antipyretic medication. The evidence summarized in the systematic review (**paper 1**) generally suggested that risks associated with prenatal fever exposure were significantly attenuated if the fever was treated with antipyretic drugs. These findings were reported for neural tube defects,[77,100,157] and for autism spectrum disorders and developmental delay.[88] However, another study reported that risks of neural tube defects were even higher if the fever was treated with antipyretic medications.[97] These findings suggest that antipyretic treatment in itself may be associated with adverse outcomes, which is consistent with a series of recently published studies,[116,158-162] implicating prenatal acetaminophen exposure (which is the most commonly used medication to treat fevers in pregnancy), with neurodevelopmental impairment. The potential adverse effects of prenatal acetaminophen does however not exclude that prenatal fever exposure might be harmful as well. In the DNBC, pregnant women did report use of antipyretic medications, but not whether use occurred in relation to fever episodes. It was consequently not possible to examine how treatment of prenatal fevers affected the risk of the selected neurodevelopmental outcomes addressed in this thesis. Nevertheless, such analyses would have been more relevant, if the associations between prenatal fever exposure and the outcomes of interest in this thesis had been more distinguished.

In summary, for neural tube defects, it does seem plausible that elevated maternal body temperature may be associated with increased disease risk, and that this risk can be attenuated by lowering the fever using

antipyretic medication. The possible teratogenic influence of maternal hyperthermia is supported by numerous animal studies,[70,72,73] and by the studies of prenatal exposure to other heat sources.[75-77,94] Nevertheless, fever does not seem to causally associated with longer-term and less pronounced neurodevelopmental impairment, including, but not necessarily limited to ADHD, academic performance, and psychosis-like symptoms.

Prenatal effects, genetics and postnatal environment

Another challenge to causal inference in this thesis, was to distinguish potential adverse effects of prenatal exposures from the effects of mother-child genome sharing or correlated conditions in the postnatal period of life.[163,164] For instance, in a recently published study from Denmark, the authors reported that prenatal exposure to infections was associated with decreased general cognitive ability among 161,696 young men.[114] However, parental exposure to infections prior to the pregnancy, as well as infections during childhood and adolescence were similarly related to impaired cognitive ability at conscription. This posits the question whether the associations reported in this thesis may in fact be explained partly or wholly by underlying genetic or social factors related to maternal susceptibility to infections as well as neurodevelopmental outcomes in the child. However, all analyses presented as part of this thesis were adjusted for a range of potential confounders, including socio-demographic, behavioral and health related conditions to minimize potential confounding. Secondly, given that only some infections (genitourinary infections and influenza-like illness) were related to subsequent neurodevelopmental impairment in the child, and others not, suggests that pathogen-specific effects may be more likely to account for these findings.

5.4 Strengths

The DNBC constitutes a unique source of systematically collected information about maternal fever and common infections in pregnancy. These exposures are not otherwise documented in registers, unless they require hospital admission or prescribed medical treatment. The exposures examined in this thesis are therefore likely to resemble those experienced in ordinary low-risk pregnancies. The detailed nature of data collection and size of the cohort furthermore allowed us to conduct several sub-analyses examining for instance the impact of timing of exposure and temperature of fever episodes. Such analyses have not commonly been reported previously, as many studies have been underpowered to examine these issues. This knowledge is however essential in facilitating our understanding of the possible role of these prenatal exposures in the etiology of neurodevelopmental disorders. In addition, all information about fevers and infections were collected prior to birth, suggesting that flawed recall and missing data most likely was non-differential.

Information from various sources was also used to adjust analyses for potential confounding. Socio-demographic characteristics and health information were usually extracted from registers, whereas information on lifestyle during pregnancy was based on the DNBC interviews, assuring a comprehensive range of variables to choose from, when including covariates. Data from the registers used in this thesis are generally considered to be updated and of high quality. In **paper 3** on academic performance, we furthermore used a sibling design in a sensitivity analysis, which allowed us to adjust for important maternal characteristics that were otherwise not measured. By comparing exposure-discordant siblings, we were able to examine associations of interest, while adjusting by design, for fixed family characteristics (such as maternal intelligence) and to some extent also for conditions that were non-stable, but correlated in postnatal life of siblings.[165,166]

In the study of ADHD a virtually complete follow-up was ensured by following the children in nation-wide registers. Over a period of on average 10.6 years, only 1.5% of the children were lost to follow-up due to death or emigration, thus minimizing selection bias. In the two studies of academic performance and psychosis-like experiences where loss to follow-up was more extensive, weighted analyses were conducted to correct for selective attrition. With the exception of a few findings, measures of association were generally only marginally affected by the weights, suggesting that loss to follow-up did not bias our results in any substantial manner. This was most likely due to the fact that attrition seemed to be largely independent of prenatal exposure status.

5.5 Limitations

Non-participation in the DNBC

Low participation in cohort studies is related to reduced generalizability of findings and potential bias due to selective participation. The women who participated in the in the DNBC were generally healthier and of higher socio-economic status and were consequently not entirely representative of the general female population giving birth in Denmark during that time (see section 4.5.1). Generally speaking, selective participation in cohort studies is usually not a main concern, given that the decision to participate cannot be based upon the future outcome. Nevertheless, characteristics associated with participation may correlate with the outcome of interest and some selection bias cannot be ruled out. For instance, maternal psychiatric morbidity would likely be associated with participation in the DNBC as well as with neurodevelopmental status in the child. However, in a recent study within the DNBC, non-participation was found to only marginally affect relative risk estimates for various assoications.[167] Although these findings cannot necessarily be generalized to the associations examined in this thesis, the authors note that the findings are generally reassuring. In addition, in the analyses of psychiatric outcomes (ADHD, and psychosis-like experiences) analyses were adjusted for maternal or parental history of psychiatric diseases, along with a variety of other possible confounders, to minimize this potential source of bias.

Live-birth bias

In perinatal epidemiology one particular source of selection bias stems from restricting analyses to live-born children.[168,169] This type of bias may be induced if the outcome of interest can only be ascertained after birth and when the prenatal exposure is also a cause of fetal loss, see **Figure 7**.
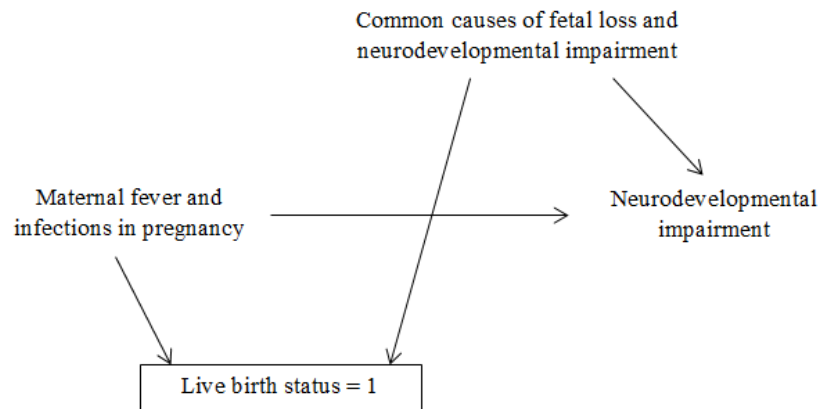
Figure 7: Bias structure in live-birth bias according to DAG principles. Restricting analyses to live-born children (Live birth status = 1), opens a bias path from pregnancy exposure to neurodevelopmental impairment through uncontrolled common causes of live birth status and neurodevelopmental outcomes. *Adapted from Liew et al.[168]*

In this thesis, live-birth bias may have affected some of our findings, given that the neurodevelopmental outcomes included in the studies are only observable among live-born children and because maternal infections are known risk factors of pregnancy loss.[170,171] However, in a simulation study, Liew and colleagues reported that the magnitude of such bias in an example of prenatal exposure to organic pollutants and subsequent ADHD in children within the DNBC, was generally small.[168] Furthermore, adjusting for common causes of the outcome and fetal loss seemed to reduce the bias. In the analyses conducted as part of this thesis, some adjustment for likely common causes of fetal loss and neurodevelopmental outcomes were done, for instance by maternal age and smoking habits in pregnancy. Also, the analyses concerning fever was probably less prone to this type of bias, given that most epidemiological studies have not found any link between fever in pregnancy and subsequent pregnancy loss,[172] also within the DNBC.[173]

Measurement of exposures

Perhaps the most important limitation of the studies presented in this thesis was the risk of bias due to measurement error. For prenatal exposures, all information was based on maternal self-report in the two pregnancy interviews. The accuracy of recall is likely to depend on a variety of circumstances, such as the amount of time that has passed since exposure, the severity of the exposure episode, the nature of the information being recalled as well as personal characteristics of the woman. For instance, flawed recall

was more likely to affect information on details of timing of exposures or temperature during fever episodes, than recall of any-time in pregnancy exposure. Similarly, the extent of misclassification of severe infections like pyelonephritis was probably less than the extent for milder infections, such as cystitis. The combination of these various influences, however, makes the magnitude and direction of the bias hard to predict.

In a supplementary quantitative bias analysis, the impact of misclassification of any-time in pregnancy exposure due to missing information on the last part of pregnancy was evaluated (see section 4.5.2). The last pregnancy interview was scheduled to take place around gestational week 30, suggesting that we on average only had exposure information on approximately 75% of the pregnancy. The bias analysis indicated that the effects only seemed to be slightly underestimated if non-differential misclassification was assumed. Even if only 50% of women that were truly exposed to fever, were correctly classified as such (i.e. a sensitivity of 0.5), then the OR changed from 1.10 in the conventional analyses to 1.18 in the corrected analysis. The magnitude of bias would most likely be similar for other prenatal exposures (i.e. the various infections) and outcomes addressed in this thesis. Bias in the analyses of any-time in pregnancy exposure due to missing information on the last part of pregnancy was consequently not a substantial problem.

Defining and measuring outcomes

Potential misclassification of the outcomes was also a concern in all three studies. In **paper 2**, we sought to minimize misclassification of ADHD status by using three nation-wide registers in combination, to detect cases of ADHD. Nevertheless, despite the comprehensiveness of this approach, identification required that the child had been prescribed medication or had at least one contact to a psychiatric department or hospital. While severe cases of ADHD most likely were identified using this approach, milder cases might not necessarily have been included. In the study of academic performance (**paper 3**), measurement error was also one of the main concerns, given that the reliability of the assessments has been questioned.[174] This was particularly critical, given that we were not able to detect any effects associated with prenatal exposure to fevers and a range of infections, which would be a problem if the precision of the assessments was too low. To overcome the uncertainty of single measurements however, we used several assessments conducted in various subjects (language and math) at different times (grades). In addition, the scores also discriminated well for other variables in our models (e.g. maternal education, smoking, stress), suggesting that the lack of associations was probably not due to the potential imprecision of test scores. Finally, bias due to misclassification of psychosis-like experiences was addressed in a supplementary analysis, using probabilistic bias analysis (section 4.5.2). Assuming nondifferentiality of misclassification for prenatal exposure status, a sensitivity of 0.5-1.0, and a specificity of 0.95- 1.0, suggested that the conventional analysis (OR=1.15) somewhat underestimated the

effects of prenatal exposure compared to the analyses corrected for misclassification (OR=1.27). It is consequently possible that our findings constitute somewhat conservative measures of the effects of fever and common infections in pregnancy.

5.6 Pregnant women, risk perception and risk communication

When all of the findings have been considered in light of their strengths and potential limitations, what is left is translating these results into meaningful knowledge for pregnant woman experiencing fevers and infections. However, increased media coverage and the growing number of scientific studies identifying biological, environmental and behavioral risk factors in pregnancy, may inadvertently create confusion, stress and anxiety among expectant mothers. Research has shown that when pregnant women perceive risk information they tend to focus more on the severity of the outcome, rather than the likelihood that it will occur.[175] This suggests that probability-based estimates, as presented in this thesis, are not necessarily transferred into meaningful concepts of risk. This provides researchers with an additional responsibility to communicate results in a careful manner. For illustrative purposes selected findings from this thesis are presented in **Table 12** using different risk measures.

Table 12: Selected associations presented using different risk measures.

Association	Frequency of outcome*	Relative risk measure	Excess cases	Proportion (%) of unexposed without outcome	Proportion (%) of exposed without outcome
1st trimester fever exposure and neural tube defects	1 in 1000 children	2.9	1.9 per 1000 exposed	99.9	99.7
Genitourinary infections during gestational week 33-36 and ADHD	2 in 100 children	1.6	1.2 per 100 exposed	98.0	96.8
Influenza-like illness during pregnancy and ≥ definite 2 psychosis-like experiences	4 in 100 children	1.5	2 per 100 exposed	96.0	94.0

 * For simplicity numbers are considered to refer to the frequency of outcome among unexposed mothers

The table illustrates that while the association between first trimester fever exposure and neural tube defects is clearly the strongest of them all, nearly all (99.7%) women that do experience a fever in early pregnancy go on to give birth to babies without neural tube defects. This is explained by the low underlying risk of having a child with this birth defect. Similarly, a vast majority of the children prenatally exposed to genitourinary infections (in late pregnancy) and influenza-like illness will also not develop ADHD nor psychosis-like symptoms. The findings reported in this thesis were generally either relatively weak or related to rare outcomes, suggesting that the overall risk of a child suffering from these neurodevelopmental consequences remain low, even if the mother is exposed while being pregnant.

Chapter 6

Conclusions

The overall aim of this thesis was to examine whether fever and common infections in pregnancy was associated with neurodevelopmental impairment in the child, specifically addressed in a systematic literature review and three separate studies on ADHD, academic performance and psychosis-like experiences.

The existing literature generally supported that fever during pregnancy may indeed have harmful consequences for the unborn child. Such adverse effects seemingly covered both short- and longer term neurodevelopmental impairment, although the evidence was more limited and inconsistent for longer-term outcomes. The strongest and most consistent finding suggested an almost 3-fold increased risk of neural tube defects, following maternal fever in first trimester.

On the basis of the three studies conducted as part of this thesis, however, prenatal exposure to fever and common infections did not or only weakly contribute to the development of ADHD, academic performance and psychosis-like experiences. We were generally not able to detect any dose-response relationship with temperature of fever episodes, and a majority of the timing analyses did not suggest that any period of the pregnancy was particular vulnerable to such exposures. A few exception were however, that a moderately increased rate of ADHD in the children was observed for maternal exposure to genitourinary infections in the last part of pregnancy and that the risks of psychosis-like symptoms also seemed to be elevated, for maternal exposure to influenza-like illness at any time of pregnancy, at least when our a priori case-definition of influenza-like illness was used.

The findings from this thesis adds substantially to the limited knowledge about the neurodevelopmental consequences in the child of prenatal exposure to fevers and common infections. Our findings suggest that these exposures do generally not contribute substantially to milder and longer-term neurodevelopmental impairment in the child. In addition, even though prenatal fevers, genitourinary infections and influenza-like illness were associated with some neurodevelopmental outcomes, all of these findings were either relatively weak or related to rare outcomes, suggesting that the overall risk of a child suffering from these consequences remain low, even if the mother is exposed while being pregnant.

6.1 Perspectives and directions for future research

The findings from this thesis particularly adds to the knowledge about how these prenatal exposures are related to one group of potential adverse health outcomes, namely those related to fetal brain development. Ultimately, studies like the ones presented in this thesis, should contribute to the formulation of recommendations and practical advice to pregnant women about the safety and potential hazards of these common conditions. Nevertheless, currently the body of evidence is not sufficient to

warrant such overall recommendations. In the meantime, however, it is important to outline what pregnant women and professionals involved in prenatal care, can make of these findings.

In the systematic review it was concluded that prenatal exposure to fever seemed to be associated with adverse health outcomes in the child, and that the effect was possibly distinct from any effect of the underlying infection. If the associations reported are in fact causal, then these findings indicate that some adverse outcomes, such as certain birth defects, can be prevented by targeting fevers in pregnancy. Strategies may include primary preventive efforts, such as vaccination against infections that are usually accompanied by fevers. For instance, in Denmark influenza vaccination is currently recommended to all women who are in their second or third trimester of pregnancy during the yearly influenza season.[176] However, on the basis of the findings from our review, it could be relevant to prevent influenza also in early pregnancy. Extending the recommendation to women planning pregnancy and those who are in their first trimester could consequently potentially reduce the proportion of women experiencing fevers in early pregnancy. In other countries (e.g. the UK and USA) influenza vaccination is recommended for pregnant women in any trimester.[177,178] Secondary preventive efforts could include treatment using antipyretic medications to lower maternal body temperature. Nevertheless, the mounting evidence implicating prenatal exposure to acetaminophen with a range of neurodevelopmental outcomes as well, complicates such recommendation. As of now, it remains unclear whether any potential risks associated with acetaminophen use in pregnancy exceeds those potentially related to fever. Important priorities for future research efforts include to establish whether a dose-response relationship with temperature exists for neural tube defects; to weigh the risks and benefits of treating fevers in pregnancy with antipyretics; and to evaluate whether other structural brain defects may by susceptible to hyperthermia induced damage as well.

Our findings also suggested that genitourinary infections in late pregnancy potentially increased the risk of ADHD in the offspring. Genitourinary infections are however generally monitored throughout pregnancy as part of the prenatal care program in Denmark, because they are known risk factors of premature birth and other adverse outcomes. Our findings consequently only reinforce the importance of continuing the existing efforts of preventing and detecting genitourinary infections as early as possible among pregnant women. Further research should nevertheless seek to establish whether genitourinary infections are in fact causally related to ADHD, or whether the observed association may be explained by associated factors such as antibiotic treatment or other pre- or postnatal conditions.

In the final study, results indicated that maternal exposure to influenza in pregnancy was possibly associated with psychosis-like experiences. Improved accuracy of determining maternal exposure status (e.g. by use of serological data) could clarify whether influenza during pregnancy is in fact creating a

susceptibility in the offspring to developing psychosis-like symptoms. Similarly, as the children within the DNBC reaches adulthood, it would be relevant to examine whether maternal influenza during pregnancy is related to clinical outcomes in the child, as well (e.g. mood disorders and psychotic disorders), and whether psychosis-like experiences during childhood may help elucidate underlying etiological mechanisms.

Finally, for fevers and many common infections in pregnancy we were not able to detect any increased risk of milder longer-term neurodevelopmental outcomes. While we cannot exclude that these exposures might constitute important risk factors for other health outcomes not addressed in this thesis, our findings generally indicate that a vast majority of children born to women experiencing fevers and common infections during pregnancy will not suffer from neurodevelopmental impairment.

7. References

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Appendix 1: Questions concerning fever and infections in pregnancy interview 1 and 2 in the DNBC

Fever

B110	Have you had fever while pregnant?

    1. yes
    2. yes, but not since the last interview ->B116
    3. no ->B116
    4. do not know ->B116
    5. do not wish to answer ->B116

B111	How many times have you had episodes of fever during pregnancy?

    1. __ times
    2. do not know
    3. do not wish to answer

B111A   Number of fever episodes (1-10)

B112	In what gestation weeks did you have the fever?

(B112**xxyy**, where xx refers to fever episode no 1, 2, 3… from B111 and yy indicates the gestation week (see master list). Variable value 0=no, 1=yes)

    B1120101-B1120142

    B1120201-B1120242

    .

    .

    B1121001-B1121042

B113_1-10   For how many days did the fever last?

    1. __ days
    2. do not know
    3. do not wish to answer

B113A_1-10 Number of days with fever (1-99)

B114_1-10   The highest temperature?

    1. ,_^{O}C
    2. did not take the temperature
    3. do not know
    4. do not wish to answer

B114A_1-10 Temperature (30,5 - 45,5)

B115	What other symptoms did you have with the fever?

For instance, did you have a headache, pain in the stomach, muscles or joints.
(B115**xxyy**, where xx refers to the fever episode from B111 and yy refers to the
answer category in B115. Variable value 0=no, 1=yes)
(Ex. B1150309 refers to stomach pains in the 3rd fever episode)

B1150101-B1151001 bladder
B1150102-B1151002 abscesses
B1150103-B1151003 diarrhea
B1150104-B1151004 cold
B1150105-B1151005 cough
B1150106-B1151006 headache
B1150107-B1151007 pain in joints
B1150108-B1151008 pain in muscles
B1150109-B1151009 pain in stomach
B1150110-B1151010 pain behind the eyes
B1150111-B1151011 sore throat
B1150112-B1151012 vomiting
B1150113-B1151013 tiredness
B1150114-B1151014 eczema
B1150115-B1151015 urinary tract symptoms
B1150116-B1151016 pain in ears
B1150117-B1151017 other_____
B1150118-B1151018 none
B1150119-B1151019 do not know
B1150120-B1151020 do not wish to answer

B115A_1-10 Other symptoms, txt

Genitourinary infections

B126	Have you had cystitis during pregnancy?

1. yes
2. no ->B128
3. do not know ->B128
4. do not wish to answer ->B128

B127	How far into the pregnancy did you have the disease?

B127_1-B127_42 (see master list)

B128	Have you had inflammation of the pelvis or kidney during pregnancy?

1. no ->B130
2. do not know ->B130
3. do not wish to answer ->B130

B129	In what gestation weeks did you have the disease?

B129_1-B129_42 (see master list)

B132	Have you had fungal infection in vagina during pregnancy?

1. yes
2. yes, but not since the last interview ->B136
3. no ->B136
4. do not know ->B136
5. do not wish to answer ->B136

B135	How far into the pregnancy did you have the disease?

B135_1-B135_42 (see master list)

Respiratory infections

B136	During the pregnancy, did you ever have coughs that lasted for more than 1 week?

1. yes
2. no ->B138
3. do not know ->B138
4. do not wish to answer ->B138

B137	In what gestation weeks did you have a long-lasting cough?

B137_1-B137_42 (see master list)

Diarrhea

B116	Have you had diarrhea during pregnancy (more than three evacuations in 24 hours)

1. yes
2. yes, but not since the last interview ->B120
3. no ->B120
4. do not know ->B120
5. do not wish to answer ->B120

B117	How many times during pregnancy have you had diarrhea?

1. __ times
2. do not know
3. do not wish to answer

B117A	Number of episodes with diarrhea during pregnancy (1-10)

B118_1-10   In what gestation weeks did you have your 1st, 2nd, 3rd….. diarrhea episode?
1. __ week
2. do not know
3. do not wish to answer B118A_1-

10 Gestation week (1-40)

B119_1-10   For how many days did you have diarrhea?
1. __ days
2. do not know
3. do not wish to answer

B119A_1-10 Number of days with diarrhea (1-99)

Persistent viral infections

B123   Have you had a cold sore/herpes in the lip during pregnancy?
1. yes
2. no
3. do not know
4. do not wish to answer

B124   Have you had a cold sore or herpes in the genitals during pregnancy?
1. yes
2. no
3. do not know
4. do not wish to answer

B125   Have you had veneral warts/ condylomata during pregnancy?
1. yes
2. no
3. do not know
4. do not wish to answer

Other infections

B120   Have you had any childhood diseases or diseases with skin rash during the
pregnancy?
1. yes
2. no ->B123
3. do not know ->B123
4. do not wish to answer ->B123

B121	What childhood disease?

B121_1 rubella
B121_2 measles
B121_3 chicken pox
B121_4 mumps
B121_5 parvovirus B19
B121_6 other _____
B121_7 do not know
B121_8 do not wish to answer

B121A	Other childhood disease, txt

B122	How far into the pregnancy did you have (answer in *B121)*?

(B122**xxyy**, where xx refers to the answer in B121 and yy indicates the gestation week (see master list). Variable value 0=no, 1=yes)

B1220101-B1220142
B1220201-B1220242

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B1220601-B1220642

From B138 to B139 runs in a loop of max.10. That means that when you reach to B139, you must start all over again asking B138 until the answer is no longer yes, or until the loop has run 10 times. The variables are named with the loop number at the end (ex. B138_3 means that it is the third time B138 is being asked)

B138_1-10	Have you had inflammations or infections during pregnancy?

1. yes
2. no ->B140
3. do not know ->B140
4. do not wish to answer ->B140

B138A_1-10	What was the name of the inflammation/infection?

1. name _____
2. do not know
3. do not wish to answer

B138B_1-10 Name of inflammation/infection, txt

B139	In what week of gestation did you have the inflammation/infection? (B139**xxyy**,

where xx refers to the loop number and yy indicates the week of gestation (see master list). Variable value 0=no, 1=yes)

B1390101-1390142
B1390201-1390242

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B1391001-B1391042