From the Department of Medical Epidemiology and Biostatistics
Karolinska Institutet, Stockholm, Sweden

WHAT CAN GENETICALLY INFORMATIVE DESIGNS ADD TO THE UNDERSTANDING OF ADHD?

Qi Chen

Karolinska Institutet

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WHAT CAN GENETICALLY INFORMATIVE DESIGNS ADD TO THE UNDERSTANDING OF ADHD?

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Qi Chen

Principal Supervisor:
Associate professor Henrik Larsson
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Co-supervisor(s):
Associate professor Arvid Sjölander
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Ralf Kuja-Halkola
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Opponent:
Professor Camilla Stoltenberg
Norwegian Institute of Public Health

Examination Board:
Associate professor Shahram Bahmanyar
Karolinska Institutet
Department of Medicine, Solna

Associate professor Lisen Arnheim Dahlström
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Associate professor Karin Brocki
Uppsala University
Department of Psychology

“Problems that remain persistently insoluble should always be suspected as questions asked in the wrong way.”

— Alan W. Watss

ABSTRACT

Attention-deficit/hyperactivity disorder (ADHD) is a highly prevalent and clinically heterogeneous neurodevelopmental disorder affecting approximately 3.4–7.3% children and adolescents and 2.5–3.4% adults worldwide, leading to adverse consequences for affected individuals, their families, and society at large. Despite the growing body of research, the etiology of ADHD remains obscure. Epidemiologic research is crucial for detecting disease risk factors. However, prior studies have rarely moved beyond identifying associations between proposed risk factors and later outcomes to rigorously testing the causality of these associations. The stagnation is partly due to the intrinsic limitations of observational studies, such as insufficient adjustment for genetic confounding. Large register-based data enable researchers to incorporate classical epidemiologic designs into genetically informative samples. Such genetically informative study designs are useful tools for unraveling the role of genetic and environmental factors in the development of diseases. In this thesis, the four studies used genetically informative designs to enhance the current understanding of the etiology of ADHD and the mechanisms underlying the associations between ADHD and its adverse health outcomes, including high body mass index (BMI) and suicidal behavior.

In Study I, we attempted to quantify the familial aggregation of ADHD in a large representative Swedish family sample, consisting of siblings and cousins of varying degrees of genetic relatedness. The results demonstrated that the familial aggregation of ADHD generally increased with increasing genetic relatedness between family members. Persistence of ADHD into adulthood in family members was associated with higher degree of the familial aggregation compared to remission of ADHD prior to adulthood.

In Study II, we assessed the co-aggregation of ADHD and overweight/obesity within families and further tested the hypothesis that the familial co-aggregation of the two conditions is at least in part due to common familial causes. The findings suggested an etiological overlap between ADHD and overweight/obesity.

In Study III, we investigated the association between high maternal pre-pregnancy BMI and offspring ADHD both at the population level and within siblings. The findings indicated the contribution of shared familial factors to the association, which supported the conclusion from Study II. A direct causal effect of high maternal pre-pregnancy BMI on offspring ADHD was, however, not evident.

In Study IV we examined the association between the use of ADHD medication and concomitant suicidal behavior. When the same individual was compared with him of herself for the rate of suicide-related events during on- and off-treatment periods, the results did not support that the use of ADHD medication increased the risk of concomitant suicidal behavior.

In conclusion, observational studies using genetically informative designs may provide valuable insights into the etiology of ADHD. The familial aggregation of ADHD increases with increasing genetic relatedness. ADHD and overweight/obesity share etiological factors.

In family settings, such shared etiological factors lead to the familial co-aggregation of the two conditions. Moreover, the shared etiological factors manifest as unmeasured familial confounding, which may partly account for the population-level association between high maternal pre-pregnancy BMI and offspring ADHD. The use of ADHD medication does not appear to increase the risk of concomitant suicidal behavior. Genetically informative designs should be increasingly used in future epidemiologic research to efficiently control for confounding and strengthen causal inferences.

LIST OF SCIENTIFIC PAPERS

I. **Chen Q**, Brikell I, Lichtenstein P, Serlachius E, Kuja-Halkola R, Sandin S, Larsson H. Family Aggregation of ADHD. (Submitted)

II. **Chen Q**, Kuja-Halkola R, Sjölander A, Serlachius E, Cortese S, Faraone SV., Almqvist C, Larsson H. Etiological overlap of ADHD and Obesity. (Submitted)

III. **Chen Q,** Sjolander A, Langstrom N, Rodriguez A, Serlachius E, D'Onofrio BM, Lichtenstein P, Larsson H. Maternal pre-pregnancy body mass index and offspring attention deficit hyperactivity disorder: a population-based cohort study using a sibling-comparison design. Int J Epidemiol. 2014;43(1):83-90.

IV. **Chen Q,** Sjolander A, Runeson B, D'Onofrio BM, Lichtenstein P, Larsson H. Drug treatment for attention-deficit/hyperactivity disorder and suicidal behaviour: register based study. BMJ. 2014;348:g3769

RELATED PUBLICATIONS

(*not included in this thesis*)

I. Skoglund C, **Chen Q**, Franck J, Lichtenstein P, Larsson H. Attention-deficit/hyperactivity disorder and risk for substance use disorders in relatives. Biol Psychiatry. 2015;77(10):880-6.

II. Ljung T, **Chen Q**, Lichtenstein P, Larsson H. Common etiological factors of attention-deficit/hyperactivity disorder and suicidal behavior: a population-based study in Sweden. JAMA psychiatry. 2014;71(8):958-64.

III. Skoglund C, **Chen Q**, D'onofrio Bm, Lichtenstein P, Larsson H. Familial confounding of the association between maternal smoking during pregnancy and ADHD in offspring. J Child Psychol Psychiatry. 2014;55(1):61-8.

CONTENTS

LIST OF ABBREVIATIONS

ADHD	Attention-deficit/hyperactivity disorder
ATC	Anatomical Therapeutic Chemical
BMI	Body mass index
CDR	Cause of Death Register
CI	Confidence interval
CNV	Copy number variant
DAG	Directed acyclic graph
DSM	Diagnostic and Statistical Manual of Mental Disorders
FDA	Food and Drug Administration
GWAS	Genome-wide association study
HKD	Hyperkinetic disorder
HR	Hazard ratio
ICD	International Classification of Diseases and related health problems
LISA	Longitudinal Database for Health Insurance and Labor Market Studies
MBR	Medical Birth Register
MGR	Multi-Generation Register
MSCR	Military Service Conscription Register
NBHW	National Board of Health and Welfare
NPR	National Patient Register
OR	Odds ratio
Pastill	Clinical Database for Child and Adolescent Psychiatry in Stockholm
PDR	Prescribed Drug Register
PIN	Personal identity number
RCT	Randomized controlled trial
rGE	Gene-environment correlation
SNP	Single nucleotide polymorphism
STR	Swedish Twin Register
TPR	Total Population Register
WHO	World Health Organization

1 INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) or hyperkinetic disorder (HKD) is a common neurodevelopmental disorder characterized by developmentally inappropriate levels of inattentive and/or hyperactive-impulsive symptoms that are manifested in multiple settings.[1] The core symptoms of ADHD have early onset in childhood and may persist into adulthood in a substantial proportion of affected individuals.[2] ADHD is associated with impairments in social, academic, and occupational functioning, leading to adverse consequences for affected individuals, their families, and society at large.[3] Despite the growing body of research, the etiology of ADHD remains obscure.

Large register-based data enable methodological innovations such as genetically informative designs. Such study designs, incorporating classical epidemiologic designs into genetically informative samples, are useful tools for investigating the role of genetic and environmental factors in the development of diseases. Demonstrating familial aggregation of a disease often serves as a crucial first step leading towards further identification of disease risk factors shared by family members. A comprehensive evaluation of the familial aggregation of ADHD in a representative Swedish sample with prospective follow-up is therefore warranted. Assessing familial co-aggregation of diseases may uncover the etiological overlaps between these diseases. For instance, if ADHD and high body mass index (BMI) indeed share etiological underpinnings, a clearer understanding of either one of the two conditions may be implicated in the preventive, diagnostic, therapeutic processes of both.

Many observational studies cannot move beyond identifying associations between proposed risk factors and diseases to rigorously testing the causality of these associations. Such stagnation is partly due to the intrinsic limitation of observational studies with respect to adjustment for unmeasured confounders. A potential association between high maternal pre-pregnancy BMI and offspring ADHD has been proposed, but the role of familial confounding in the association needs to be clarified before any causal claim can be made. Likewise, pharmacoepidemiologic studies of the association between the use of ADHD medication and suicidal behavior may be confounded by ADHD per se being associated with suicidal behavior via genetic overlap. Genetically informative designs (e.g. sibling comparison and within-individual comparison) can be used to efficiently control for certain types of unmeasured confounders, including, but not limited to, genetic background, and therefore may help address the causal nature of the aforementioned associations.

This thesis seeks to illustrate the applicability of genetically informative designs in observational studies that aim to advance the understanding of the etiology of ADHD and the mechanisms underlying the associations between ADHD and its adverse health outcomes.

2 BACKGROUND

2.1 ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD)

2.1.1 Early history

Despite the ongoing debate over whether ADHD is a valid diagnosis, the ADHD-like syndrome is not a contemporary phenomenon. The earliest medical literature on ADHD dates back to 1775 when the German physician, Melchior Adam Weikard, first described a series of inattentive symptoms in a German textbook.[4] This was 33 years before the term "psychiatry" was first coined in human history. In 1789, similar inattentive symptoms caught the attention of a Scottish physician, Sir Alexander Crichton, whose written account denotes "mental restlessness".[5] Surprisingly, his description of the symptoms, though not fully meeting the criteria for a clinical diagnosis, was found to be almost entirely consistent with the symptoms of the inattentive subtype of ADHD in the *Diagnostic Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (DSM-IV-TR)[6] released two centuries later.

2.1.2 Clinical Diagnosis

Two classification systems have been used in parallel for the diagnosis of ADHD/HDK: the DSM in the United States and the *International Statistical Classification of Diseases and Related Health Problems* (ICD) in Europe and other parts of the world. The DSM-IV allows for an ADHD diagnosis based on the presence of either inattentive or hyperactive/impulsive symptoms, together with impairments in social, academic, and/or occupational functioning caused by the symptoms.[6] ICD-10 diagnostic criteria for HKD require the presence of both inattentive and hyperactive/impulsive symptoms across more than two settings (e.g. school and home).[7] Compared with the DSM-IV, the ICD-10 is more restrictive and tends to capture individuals affected by more severe forms of the disorder.[8] Since 2013, the DSM-5 has replaced the DSM-IV.[9] Major changes in the DSM-5 diagnostic criteria for ADHD are as follows: (1) the age of onset is changed from before 7 years to before 12 years; (2) only five instead of six symptoms for each symptom domain are required for the diagnosis among individuals 17 years or older; and (3) ADHD is allowed to be diagnosed in conjunction with autism spectrum disorder.[10,11] Apart from these major changes, the DSM-5 has retained the three categories of ADHD from the DSM-IV, which are now referred to as combined presentation, predominantly inattentive presentation, and predominantly hyperactive-impulsive presentation.[9]

2.1.3 Prevalence

The worldwide prevalence of ADHD has been estimated to be 3.4–7.2% in children and adolescents.[12,13] The large variation in the prevalence estimates results mainly from methodological heterogeneity across studies conducted in different geographic locations.[14,15] The most influential methodological factors include selection of diagnostic criteria, source of information, and requirement of functional impairment for a diagnosis to be made.[14,15] In

recent years, the rate of clinical diagnosis of ADHD in children and adolescents is increasing,[16] most likely owing to improved public awareness, more accessible health care, and changing clinical practices. However, there is no solid evidence supporting an increase in the real prevalence of ADHD over the past three decades.[14] Despite common concerns about over-diagnosis of ADHD in children, systematic over-diagnosis is not evident.[17] Although ADHD is over-represented among males in clinical settings, no sex difference in the prevalence of ADHD was found between non-referred males and females.[18] Longitudinal studies have reported higher rates of age-dependent symptomatic decline for hyperactivity/impulsivity than for inattention.[19,20] Approximately two-thirds of the patients cannot achieve full remission by adulthood and 15% of patients continue to meet full diagnostic criteria for ADHD.[2]

The prevalence of adult ADHD has been estimated to be 2.5–3.4%.[21,22] With the implementation of the DSM-5, it is likely that more adults will be diagnosed with ADHD.[11,23] Nevertheless, adults with ADHD remain largely under-diagnosed and untreated.[24] Currently, a valid diagnosis of adult ADHD requires childhood-onset of ADHD symptoms.[9] One cohort study, however, has shown that 90% of adult ADHD cases did not have childhood onset of symptoms, challenging the current diagnostic conventions.[25]

2.1.4 Pathophysiology

Neuropsychological functioning and neuroimaging research has revealed the neurobiological heterogeneity of ADHD. Deficits in multiple neurocognitive domains related to ADHD have been reliably identified. The most replicated findings point to executive functioning deficits, including deficits in working memory, response inhibition, vigilance, and planning.[26] Studies of delay aversion have shown that patients with ADHD are inclined to prefer smaller immediate rewards to larger delayed ones.[27,28] Deficits in other functioning domains, including reaction time variability,[29] language and speech,[30] and motor control,[31] have also been linked to ADHD. While most patients with ADHD suffer from impairment in one or two, but rarely all, of these neurocognitive domains, some patients show no impairment in any domain.

Neuroimaging studies have revealed possible associations between ADHD and multiple structural and functional brain abnormalities. Functional magnetic resonance imaging studies have highlighted that alterations in neural networks may be implicated in ADHD in both children and adults.[32] Specifically, hypoactivity in the frontoparietal and ventral attentional networks and hyperactivity in the default, ventral attention, and somatomotor networks have been observed in pediatric patients, while hypoactivity in the frontoparietal system and hyperactivity in the visual, dorsal attention, and default networks have been found to be predominant in adult patients. Structural magnetic resonance imaging studies have linked changes in the basal ganglia and limbic regions to ADHD and indicated that these changes might become less obvious over time among individuals treated by ADHD medication.[33]

2.1.5 Risk factors

ADHD is a multifactorial disorder influenced by the complex interplay between many genetic and environmental factors within their temporal context. To date, no biomarker has been identified for screening the risk of the disorder or facilitating the diagnostic procedure. Despite a lack of thorough understanding of the etiology, studies have shown that ADHD is better viewed as an extreme of a continuously varying quantitative trait in the population and the full range of ADHD symptoms seem to be affected by the same genetic and environmental factors.[34]

2.1.5.1 Genetic factors

Family studies have reported familial aggregation of ADHD in first- and second-degree relatives.[35-38] Twin studies have estimated the relative genetic and environmental contributions to the phenotypic variance in ADHD in the population. The heritability of ADHD, i.e., the proportion of phenotypic variance in the quantitative trait or the liability of ADHD that is attributable to genetic variation in the population, is around 70-80%.[39,40] This heritability estimate is almost as high as the estimate for schizophrenia.[41] With the advent of genomic era, molecular genetic studies are beginning to reveal the complex genetic architecture underpinning the disorder. About 40% of the heritability can be attributed to common genetic variants, particularly single nucleotide polymorphisms (SNPs), of small individual effects.[42] Although genome-wide association studies (GWASs) have failed to identify specific genome-wide significant loci associated with ADHD,[43] meta-analyses of candidate gene studies have suggested associations between genetic variants involved in monoamine neurotransmitter system and ADHD.[44] GWAS-based pathway and network analyses have linked ADHD to genes regulating the dopaminergic, serotonergic, and noradrenergic systems and neuritis outgrowth.[45,46] Large rare copy number variants (CNVs) appear to play a role in the etiology of ADHD.[47-49] These CNVs are also implicated in schizophrenia and autism.[48]

In addition, common psychiatric disorders, including ADHD, are found to be influenced by a general genetic factor.[50] Prior studies of familial co-aggregation of two disorders have indicated etiological overlaps of genetic origin between ADHD and other psychiatric conditions including schizophrenia,[51] bipolar disorder,[51] substance use disorder,[52] and suicidal behavior.[53] Much less is known about the relationship between ADHD and non-psychiatric conditions such as obesity.

2.1.5.2 Non-genetic factors

Observational studies have linked ADHD to several putative non-genetic risk factors, including prenatal and perinatal conditions, toxins, dietary factors, and psychosocial adversities,[54] with the causal nature of these factors being largely unknown. Family-based quasi-experimental studies (e.g. co-twin control, sibling- and cousin-comparison, and adoption studies) enable testing of the extent to which these associations are consistent with causal hypotheses.[55] According to the findings from such genetically sensitive studies, several

observed associations between prenatal and perinatal factors (e.g. smoking during pregnancy and stress during pregnancy) and ADHD are better explained by unmeasured familial confounding.[56-58] In contrast, low birth weight, advanced paternal age at childbearing, low family income, and severe psychosocial deprivation might be causal risk factors of ADHD.[59-62]

In recent years, maternal pre-pregnancy overweight/obesity has been proposed as risk factor for neurodevelopmental outcomes, including ADHD, in offspring later in life.[63,64] These proposed associations are biologically plausible given the well-known fetal programming hypothesis, i.e., exposure to suboptimal intrauterine environment may alter physiological parameters during the development of embryo and such physiological alternations may result in long-term adverse effects on offspring development.[65] Two epidemiologic studies have reported associations between pre-pregnancy overweight/obesity and teacher-rated ADHD symptoms among school-aged children.[66,67] The findings were further supported by an animal study in a randomized experimental setting.[68] However, considerable inter-species differences largely limited the generalizability of findings from animals to humans.[69] Drawing a causal conclusion based on the animal study would be premature, especially when the causal hypothesis has not yet been tested in family-based quasi-experimental studies.

2.1.6 Adverse health outcomes

ADHD has been associated with many detrimental health outcomes (e.g., substance use, sleeping problems, obesity and physical fitness, suicide, and premature mortality).[70,71] The associations with obesity and suicide are two relevant themes in this thesis.

2.1.6.1 Associations with obesity

Obesity is defined as excessive fat accumulation in adipose tissue, leading to impaired health.[72] The most commonly used measure for overweight and obesity is BMI. BMI is calculated as body weight in kilograms divided by height in meters squared, and categorized into under weight (BMI<18.5), normal weight (18.5≤BMI<25), overweight (25≤BMI<30), and obesity (BMI≥30) according to the criteria given by the World Health Organization (WHO).[73] During the last few decades, many countries have documented an increase in the average BMI at the population level.[74-76] The prevalence of obesity in adults is estimated to be more than 10% in Sweden[75] and 34.9% in the United States.[77] Given its adverse impact on health and quality of life, obesity imposes considerable economic burden to individuals and public health systems.[78] Despite meta-analytic evidence for significant association between ADHD and obesity in children and adults,[79] the underlying mechanisms are yet to be fully understood.

Several plausible explanations might account for the association between ADHD and obesity. First, ADHD might directly cause obesity through the inattentive and impulsive components.[80] For example, lack of self-awareness of food intake due to inattention and deficient inhibitory control may give rise to unhealthy eating behaviors, which may, in turn, lead to increased risk of obesity. Life style factors may serve as mediators of the association.

Studies have reported that children with ADHD tend to spend more time in watching television and engage in less physical activity compared with children without ADHD.[81] Second, ADHD and obesity may share common etiological factors. Reward deficiency, which results from dysfunction of dopaminergic system, and oxidative stress have been proposed as the neurobiological mechanisms underlying the etiology of ADHD and obesity.[82,83] Moreover, ADHD and obesity are both highly heritable complex conditions [39,40,84] and common pleiotropic genetic variants might regulate both traits.[85] Third, individuals with obesity might have sleeping and breathing problems and thereby manifest ADHD-like symptoms.[82] In this thesis, however, we assumed that ADHD diagnosis due to such symptomatic confusion was unlikely because the ascertainment of ADHD cases was primarily based on clinical diagnosis made by specialists in psychiatry rather than community surveys.

2.1.6.2 Association with suicidal behavior

Suicide is one of the leading causes of death worldwide. The estimated worldwide annual mortality is 11.4 per 100 000 population (15.0 for males and 8.0 for females).[86] In most countries, males are more likely to commit suicide than females, whereas females attempt suicide more frequently than males.[87] In recent years, suicide rates have been increasing among young men.[88] Furthermore, it has been shown that suicide aggregates in families.[89,90] Convergent evidence from adoption, twin, and family studies consistently suggests a genetic contribution to suicidal behavior. The heritability of nonfatal suicide attempts varies between 17% and 45%.[91] Although rare in the general population, suicide rates are higher in individuals with psychiatric conditions, particularly mood disorder and substance use disorder.[92] Growing evidence has shown that ADHD is associated with an increased risk of suicidal behavior.[93-96] One Swedish population-based family study has found that ADHD and suicidal behavior shared etiological factors.[53] The shared etiological factors might have roots in serotonergic system; for instance, low concentration of cerebrospinal fluid 5-hydroxyindoleacetic acid has been implicated in impulsive suicidal behavior.[97]

2.1.7 Treatment

Comprehensive treatment of ADHD involves patient education, non-pharmacotherapy, and pharmacotherapy modalities.[1] Education to patients with ADHD and their families may improve adherence to treatment and help optimize the effects of non-pharmacotherapy and pharmacotherapy.[98] Cognitive behavioral therapy is the most commonly used non-pharmacologic option for ADHD. It also constituted a key component of the Multimodal Treatment study of children with ADHD (MTA).[99] From the MTA study, no obvious additional benefit from combining non-pharmacotherapy and pharmacotherapy over pharmacotherapy alone was observed in terms of core symptom reduction. However, the combination helped with the relief of secondary symptoms and the improvement of functioning.

Pharmacotherapy remains a cornerstone of ADHD treatment. According to the treatment recommendations from the Swedish Medical Products Agency, pharmacotherapy should be reserved for moderate to severe cases, or mild cases for which non-pharmacologic options have failed.[100] In Sweden, registered medications for ADHD treatment include psycho-stimulants (i.e., methylphenidate, amphetamine, and dexamphetamine) and the non-stimulant (i.e., atomoxetine). Both psycho-stimulants and atomoxetine have shown beneficial effects on the core symptoms of ADHD.[101] Psycho-stimulants increase intra-synaptic dopamine and noradrenaline concentration.[102] Methylphenidate is a pre-synaptic reuptake inhibitor of dopamine and noradrenaline and is recommended as first-line pharmacotherapy for ADHD,[103] whereas amphetamine and dexamphetamine also increase the release of dopamine in the pre-synaptic neuron. Meta-analytic evidence supports the efficacy of stimulants for ADHD in both children and adults.[104] Common adverse effects of stimulants include headache, decreased appetite and sleep disturbance.[105] Small delays in growth may occur to some individuals, but these delays hardly influence ultimate height in adulthood.[106] Atomoxetine acts as a selective noradrenaline reuptake inhibitor. Since atomoxetine does not increase the risk for abuse and has generally lower risk of other side effects compared to stimulants, it is considered a safe option for the treatment of comorbid ADHD and substance abuse disorder.[107] For patients with comorbid psychiatric conditions, particularly severe mood or substance use disorder, effective management of ADHD can be achieved after these comorbid conditions are treated first.[108]

Suicide risk assessment is a critical part of psychiatric evaluation. Although suicide-related events rarely occur with pharmacotherapy of ADHD, cautions about suicidality are added to the information on product characteristic of methylphenidate and dexamphetamine.[109] Based on 12 short-term, placebo-controlled clinical trials, the Food and Drug Administration (FDA) issued a black-box warning on atomoxetine due to a small but significantly increased risk of suicidal ideation in children and adolescents in 2005.[110] A meta-analysis of 14 clinical trials also reported a statistically significant association between the use of atomoxetine and suicidal ideation.[111] Small observational studies have shown that medicated ADHD patients had increased risk of suicidal behavior compared with the general population.[112,113] In contrast, one study reported that boys treated with methylphenidate had decreased risk of suicide attempts later in young adulthood compared with those untreated.[114] The rarity of suicide-related events (completed suicide or suicide attempts) in prior studies raised substantial uncertainty surrounding the results. It remains unclear whether the possible treatment-emergent suicidal ideation suggested by the clinical trials will diminish as the treatment proceeds or eventually develop into suicidal behavior.

2.2 CAUSAL INFERENCE IN BRIEF

Scientific knowledge can be viewed as a body of currently unrefuted hypotheses that seem to explain existing observations.[115] Likewise, the goal of causal inference in epidemiologic research is not to provide conclusive evidence for causality, but to test whether a causal hypothesis can survive attempts at refutation, so as to narrow down the number of plausible

explanations for observable associations.[116] In the following text, causal inference in randomized controlled trails (RCTs) and observational studies will be briefly discussed.

2.2.1 Randomized controlled trials

RCT is deemed to provide the highest level of evidence for causal inference in medical science.[117] In RCT, eligible study participants are randomly assigned to intervention group and control group.[118] Both groups are followed by a certain length of time. Through randomization, any systematic differences between the two groups at the baseline are balanced out given a large enough sample size. Significant between-group differences at the end of the follow-up can thereby be solely attributed to the intervention. Nevertheless, RCTs are not always feasible due to operational, ethical, or economical considerations.[119] For instance, in research aiming at investigating whether pre-pregnancy obesity causes offspring ADHD, researchers cannot experimentally manipulate maternal BMI levels. Using RCTs to examine the occurrence of rare adverse events (e.g. suicide attempts) potentially related to an intervention would be very expensive due to the required large sample and long follow-up. Participants failing to comply with their allocated treatment (i.e., non-compliance) may induce systematic differences between groups that were not there at baseline.[120] Lack of external validity is another limitation of RCTs, as the eligible participants in RCTs may not be representative of the target population owing to strict inclusion and exclusion criteria.[121] Some researchers have pointed out that RCTs are no more than good experimental designs under certain circumstances and the shortcomings of RCTs can make themselves inferior to many appropriately designed observational studies.[122]

2.2.2 Observational studies

In observational studies, researchers address the potential effect of an exposure on an outcome based on a study population or a sample randomly drawn from the target population, without exposure intervention. Although definitive evidence for the causality of the exposure-outcome association is unattainable, findings from observational studies may have better real-life applicability compared to findings from RCTs.[122] In observational studies, an observed association between a putative risk factor X and an outcome Y in a population might be due to one or more of the following reasons: (1) X causes Y; (2) Y causes X (reverse causation); (3) X and Y share a common cause C (confounding effect); (4) random events.[60] Studies using prospective longitudinal designs may rule out reverse causation, because an outcome cannot cause an exposure if the outcome is preceded by the exposure. Increasing sample size may reduce the influence of random events. However, insufficient adjustment for confounding is an intrinsic limitation of observational studies.

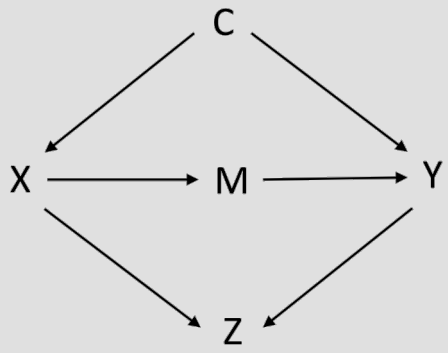
Confounding leads to a lack of comparability between exposed and unexposed groups, which gives rise to a bias in the estimation of the causal effect of an exposure on an outcome.[123] Such lack of comparability can be caused jointly by a group of risk factors of the outcome, or confounders. A confounder refers to a cause or a proxy of a cause of the outcome, which is associated with, but not affected by, the exposure.[115] For example, cigarette lighter users are

more likely to develop lung cancer, but such empirical association is not causal, because it is confounded by smoking behavior. Obviously, smokers are over-represented among cigarette lighter users and smoking is the real causal risk factor of lung cancer. In other words, smoking, as a confounder, induces a confounding bias to the association between the use of cigarette lighter and lung cancer.

Box 1. Directed acyclic graphs

Directed acyclic graphs (DAGs) have long been used to visually present hypothetic relations between variables, which may facilitate the analytic process of identifying sources of bias and help with statistical adjustment.

- An arrow between X and M means X may cause M, but not the other way around.
- The lack of arrow from C to M means that C does not have a direct causal effect on M (however, C may have a mediated effect on M through X).
- Variable M lies in the causal path way between X and Y and thus is a mediator of the causal effect of X on Y.
- Variable C is the common cause of X and Y and is referred to as a confounder. When estimating the causal effect of X on Y, we should adjust for variable C.
- Since two arrows point towards the same variable Z, Z is a collider and represents the common effect of X and Y. A spurious assotiation between X and Y may be induced by inappropriate adjustment for the collider Z, which is called collider bias or selection bias. When estimating the causal effect of X on Y, we should not adjust for variable Z.



In observational studies, confounding can be addressed through controlling for confounders.[123] Reliably measured confounders can be handled through stratification or regression model adjustment. When confounders cannot be precisely measured (e.g. genetic

background and childhood environment), genetically informative study designs may come into play.

2.3 WHY ARE GENETICALLY INFORMATIVE DESIGNS NEEDED?

Large register-based data and reliably identified family pedigrees enable researchers to incorporate classical epidemiologic study designs (e.g. cohort designs and case-control designs) into family samples or samples with measurements repeatedly taken from the same individuals in observational studies. Such genetically informative designs are superior to classical study designs with respect to elucidating the role of genetic and environmental factors in the development of diseases. There is a critical need for genetically informative designs in observational studies for several reasons.

First, they make important contribution to studies assessing familial aggregation and estimating familial risk of a specific disease. Demonstrating familial aggregation of a disease often serves as the first step leading towards further identification of genetic determinants of a disease. Under certain conditions, comparing the strength of the familial aggregation of a disease between subgroups of relatives (e.g. maternal and paternal half siblings) may shed new light on genetic and environmental contributions to the risk of the disease. Moreover, estimating familial risk of a disease by sex and disease feature may help identify target groups for diagnostic screening and enlighten counseling services. Due to limited access to representative family samples, prior studies have never comprehensively assessed familial aggregation of ADHD among family members who are of varying degrees of relatedness and nested in the sample population. A nation-wide study of the familial aggregation of ADHD, with special focus on the impacts of sex and persistence of ADHD into adulthood (Study I), is therefore warranted.

Second, given large enough family sample, familial aggregation studies can be extended to familial co-aggregation of two diseases. Familial co-aggregation studies estimate familial risk of one disease among individuals with family history of another disease. Under plausible assumptions, researchers can test whether two diseases share etiological factors or they simply represent two distinct entities. Such studies are appealing because if an etiological overlap between two diseases is confirmed, advancing the understanding of one disease may benefit the preventive, diagnostic, and therapeutic processes of both. Study II was a familial co-aggregation study seeking evidence for an etiological overlap between two highly heritable conditions, ADHD and overweight/obesity.

Third, the application of genetically informative designs may shift the field of developmental epidemiology from documenting putative associations to systematically testing rival causal hypotheses. Studies using genetically informative designs (e.g. sibling and cousin comparisons) can handle unmeasured familial confounding by design features and measured confounding in the same way as traditional epidemiologic research. Accordingly, these studies may provide more stringent causality tests. In Study III, we revisited the association

between high maternal pre-pregnancy BMI and offspring ADHD using a sibling-comparison design.

Last but not least, within-individual comparison design, as a special case of genetically informative designs, can make pharmacoepidemiologic studies less prone to confounding by indication (i.e., indication for treatment is associated with the risk of outcome under study) in the investigation of drug safety issues. In Study IV, we investigated the association between the use of ADHD medication and concomitant suicidal behavior using a within-individual comparison design to account for the potential confounding by indication due to the fact that ADHD per se is associated with suicidal behavior.[93-96]

The rationales behind the genetically informative designs used in this thesis will be further discussed in Section 5.1.

3 AIMS

The overarching aim of this thesis was to enhance the current understanding of the etiology of ADHD and the mechanisms underlying the associations between ADHD and its adverse health outcomes by using genetically informative study designs.

The specific aims of each study were:

I. To comprehensively and precisely evaluate the familial aggregation of ADHD among relatives of varying degrees of genetic relatedness and to examine the influences of sex and persistence of ADHD into adulthood on the familial aggregation (Study I)

II. To assess the familial co-aggregation of ADHD and overweight/obeisty in siblings and cousins and to test the hypothesis that ADHD and overweight/obesity share etiological factors (Study II)

III. To examine whether the association between high maternal pre-pregnancy BMI and offspring ADHD is consistent with a causal hypothesis or better explained by familial confounding (Study III)

IV. To investigate whether the use of ADHD medication can increase the risk of concomitant suicidal behavior (Study IV)

4 DATA SOURCES

4.1 SWEDISH REGISTERS

Every resident in Sweden has been assigned a ten-digit personal identity number (PIN) since 1947.[124] The PIN serves as a unique identifier for public administration and enables unambiguous linkage between national registers, which forms the basis of register-based medical research in Sweden.

The Total Population Register (TPR) was established by Statistics Sweden in 1968.[125] The register contains information on sex, date and place of birth, date of death, date of migration, civil status, and many other variables for Swedish residents who were born since 1932 and being alive in 1968.

The Multi-Generation Register (MGR) is part of the TPR and links individuals in the TPR (index persons) to their biological and adoptive parents.[126,127] Index persons (i.e., individuals through whom family relationships are ascertained) are limited to those born since 1932 and those alive on January 1, 1961. Linkages between index persons and their parents are missing if the parents were no longer alive in 1947 when PIN was initially introduced. The register has nearly complete coverage for index persons born in Sweden since 1947, among whom 97% can be linked to their biological mothers and 95% to their biological fathers. In immigrant families, such linkages are possible only if the immigrations took place before the index persons turned 18. The register enables identification of family relatives of varying degrees of relatedness. In Studies I-III, the register was used to identify siblings and cousins.

The Medical Birth Register (MBR) is kept by the National Board of Health and Welfare (NBHW). The register contains information on prenatal and perinatal variables related to nearly all pregnancies and births in Sweden since 1973, with approximately 2% complete missingness.[128] The register was used to identify birth cohorts in Study I-III and to retrieve information on maternal pre-pregnancy body weight and height as well as other covariates in Study III.

The Swedish Twin Register (STR) was established in the late 1950s and is maintained at the Department of Medical Epidemiology and Biostatistics, Karolinska Institutet. Data have been collected through multiple waves of questionnaires. The register currently contains over 75,000 twin pairs with zygosity determined via questions about intra-pair similarity and validated by genotyping data.[129] Data in this register were used to identify monozygotic and dizygotic twins in Study I.

The Swedish National Patient Register (NPR) was established and is currently held by the NBHW.[130] The register covers somatic inpatient care since 1964 and psychiatric inpatient care since 1973, with complete coverage achieved since 1987. Data on outpatient visits to specialist physicians in public care are available since 2001, even though the coverage is limited to approximately 80%. Information on primary care or other care services provided by non-physicians (e.g., psychologists and physiotherapists) is missing. For each patient, the

register contains discharge dates as well as primary and secondary diagnoses coded according to the ICD-8 (1969 –1986), ICD-9 (1987 – 1996) and ICD-10 (1997 – 2009). This register was used to identify ADHD cases (Study I-IV), suicide attempts (Study IV), and other psychiatric diagnoses as covariates (Study I, II and IV).

The Prescribed Drug Register (PDR) covers information on all drugs prescribed and dispensed in ambulatory care to the entire population in Sweden since July 1, 2005.[131] It contains data on active ingredients coded according to the anatomical therapeutic chemical (ATC) classification system, dispensed amount and dosage, prescribing and dispending dates, etc. The register was used to identify individuals who received prescriptions of ADHD medications in Study I–III and to define the time-varying treatment status by ADHD medications in Study IV.

The Clinical Database for Child and Adolescent Psychiatry in Stockholm (Pastill) includes data on diagnoses according to the DSM-IV or the ICD-10 and dates of diagnoses from Child and Adolescent Mental Health Services in Stockholm County since 2001.[132] The database was used to identify ADHD cases according to the DSM-IV and the ICD-10 diagnostic criteria (Study I-III).

The Military Service Conscription Register (MSCR) was introduced in 1901. It contains data on physical and psychological variables measured at age 18-20 for Swedish men who were conscripted for military service. In Sweden, military conscription was mandatory for all male citizens until 2007, with approximately 2–3% being exempted, mainly owing to severe handicaps or congenital disorders.[133] In 2009, the obligatory military service was replaced by voluntary military service. The register was used to identify body weight and height of index persons in Study II.

The Cause of Death Register (CDR) was established in 1952 and is updated annually. Complete coverage of the register has been achieved since 1961. The register contains mandatorily reported information on principle and contributing causes of deaths, coded according to the ICD system for all diseased individuals who were registered as living in Sweden at the time of death.[134] The register was used to identify individuals who died by suicide (Study IV) and to define censoring points during the follow-ups (Study I, III, and IV).

Longitudinal integration database for health insurance and labor market studies (LISA) was established in 1990 and is currently held by Statistics Sweden.[135] The database is annually updated and contains information on, for example, highest level of education, unemployment, social benefits, and family income for all Swedish residents aged 16 years or older. Data on highest level of education (Study II and III) was retrieved from this register.

4.2 MAIN MEASURES

4.2.1 ADHD

ADHD cases were defined as individuals who received a diagnosis of HKD (ICD-9: 314; ICD-10: F90) according to the NPR, or a diagnosis of HKD and/or ADHD (DSM-IV: 314) according to Pastill, or a drug prescription of ADHD medication [i.e., methylphenidate (N06BA04), amphetamine (N06BA01), dexamphetamine (N06BA02), or atomoxetine (N06BA09)] according to the PDR during each study period. In Study I, information on ADHD diagnosis (a drug prescription was also based on a diagnosis) was used to define a time-varying exposure. In Study I and III, attained age at first-trackable ADHD diagnosis was used as a time-to-event outcome. In Study II, ADHD status was treated as a binary outcome (presence/absence). In Study IV, ADHD cases identified exclusively from the NPR constituted the study cohort.

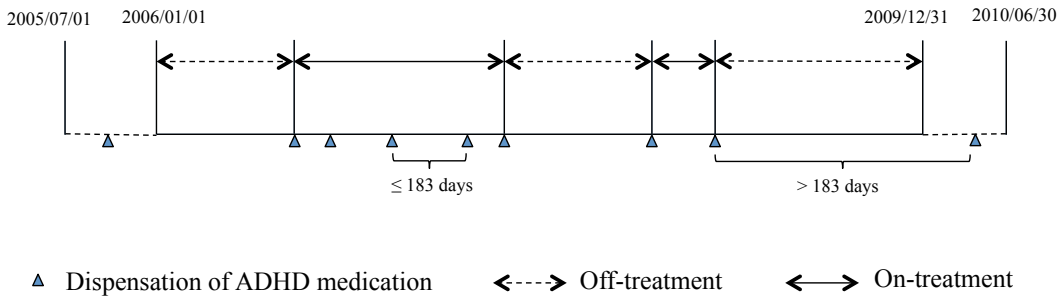
4.2.2 BMI

In Study II, data on objectively measured body weight and height were retrieved from the MSCR.[136] In Study III, information on self reported height and objectively measured weight at the first antenatal visit (around gestational week 10) were extracted from the MBR.[137] In both studies, BMI was calculated as body weight in kilograms divided by height in meters squared, and subsequently categorized into underweight (BMI<18.5), normal weight (18.5≤BMI<25), overweight (25≤BMI<30), and obesity (BMI≥30) according to the criteria given by the WHO.[73] Categorical BMI was used as an exposure in both Study II and III.

4.2.3 Treatment status by ADHD medications

Treatment status by ADHD medications was treated as a time-varying exposure in Study IV. Specifically, for each patient, we divided the entire follow-up from January 1st, 2006 to December 31st, 2009 into on- and off-treatment periods (Figure 4.1). An on-treatment period was defined as a time interval that covered a sequence of dispensations of ADHD medications, without any gap of more than 6 months (183 days) between two consecutive dispensations. The time periods not occupied by the on-treatment periods were defined as off-treatment periods. The start of an on-treatment period was the date of the first dispensation and the end of the on-treatment period was the date of the last dispensation. We took dispensations in 2005 and 2010 into account when defining the treatment status over the first and the last periods during the follow-up (Figure 4.1).

Figure 4.1: Treatment status by ADHD medications as a time-varying exposure



4.2.4 Suicide-related events

In Study IV, suicide-related events were defined as attempted or completed suicides according to ICD-10 codes (X60-X84: intentional self-harm; Y10-Y34: event of undetermined intent) and identified from the NPR and the CDR, along with the dates of their occurrences.

5 METHODS

5.1 GENETICALLY INFORMATIVE DESIGNS

5.1.1 Familial aggregation and co-aggregation studies

Familial aggregation studies are observational studies that assess the clustering of disease in family members due to shared genetic or environmental factors or both. Genetic origin is often assumed to be responsible for the clustering of disease if the change in the genetic relatedness between family members is mirrored by the change in the disease risk in the family members.[138]

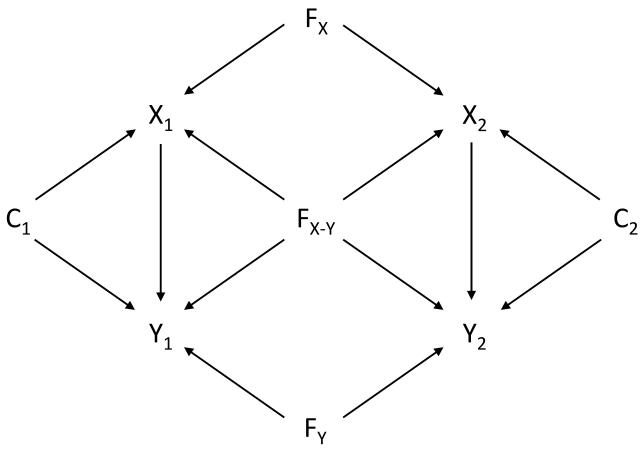
In a familial aggregation study, researchers estimate disease risk in study participants given family history of the same disease. One way to look at family history of a disease is that it serves as a proxy of the genetic susceptibility of a disease. Whether a participant is genetically susceptible to a disease is set at birth, but is considered unknown until at least one of his or her family members is diagnosed with the disease. The ascertainment of the genetic susceptibility can be carried out retrospectively via measuring the disease status of one or more close family members of the participant. For instance, when studying the familial aggregation of ADHD in first-degree relatives (i.e., parents, siblings, and offspring), researchers may be solely interested in the association between ADHD among index persons and ADHD among the relatives of the index persons, regardless of whether the index persons receive ADHD diagnosis before or after their relatives do. In this setting, it is logical to view the disease status, both for index person and relative, as a binary variable, and analyze the data in, for instance, logistic regression models. However, family members of a diseased individual may be more likely to seek medical care and receive diagnosis, possibly owing to increased awareness and concern about the disease. Accordingly, familial risks can be calculated using survival models to accommodate the time-varying effects of the disease status of index person on the risk of the same disease in his or her family members so as to provide estimates with better real-world implications in terms of disease risk prediction.

Familial aggregation studies can be extended to familial co-aggregation of two diseases. In familial co-aggregation studies, the research interest lies in estimating the risk of one disease in study participants given family history of another. An example is a study of the association between ADHD among index persons and the suicide attempt among relatives of the index persons. Hudson JI *et al.* proposed a structural approach to study the familial co-aggregation of two diseases using DAGs.[139] According to this approach, it is possible to test whether two diseases share etiological factors under certain assumptions.

Figure 5.1 illustrates the rationale behind familial co-aggregation studies. In this DAG, F_X is a latent factor representing familial causes unique for disease X. $F_{X\text{-}Y}$ is a latent factor denoting common familial causes for disease X and Y. Estimating familial co-aggregation of disease X and Y is to estimate the association between disease X in relative No.2 (X_2) and disease Y in relative No.1 (Y_1), which can be attributed to the open paths $X_2 \leftarrow F_X \rightarrow X_1 \rightarrow Y_1$

and $X_2 \leftarrow F_{X\text{-}Y} \rightarrow X_1$. It is noteworthy that the association exists only if at least one of F_X and $F_{X\text{-}Y}$ is present. The hypothesis test for an etiological overlap between disease X and disease Y can be viewed as seeking evidence for the presence of $F_{X\text{-}Y}$. A valid hypothesis test (i.e., a test of the presence of $F_{X\text{-}Y}$ that is not contaminated by the presence of F_X) requires additional assumptions, such as an absence of a direct within-individual effect of disease Y on disease X and positive associations represented by all arrows.[139] The plausibility of the assumptions largely depends on research question, researchers' subject matter knowledge, and data quality.

Figure 5.1: Directed acyclic graph for familial aggregation and co-aggregation studies.
Figure modified from Hudson JI *et al.*[139]



X_1: Disease X in relative No.1; X_2: Disease X in relative No.2; Y_1: Disease Y in relative No.1; Y_2: Disease Y in relative No.2; Y_2: Disease Y in relative No.2; $F_{X\text{-}Y}$: Common familial causes for Disease X and Y; F_X: Familial causes unique for Disease X; F_Y: Familial causes unique for Disease Y; C_1: Common causes for Disease X and Disease Y specific for relative No.1; C_2: Common causes for Disease X and Disease Y specific for relative No.2; Relative No.1 and Relative No.2 can be first-, second-, or third-degree relatives.

In register-based settings, disease status is often ascertained via registered clinical diagnosis and treated as a binary variable (presence or absence). However, the aforementioned approaches can also be applied to investigate familial aggregation or co-aggregation of certain behavioral traits or physiological conditions.

5.1.2 Quasi-experimental studies

Quasi-experimental studies are studies evaluating the impact of an exposure on an outcome without random assignment of study participants to exposed and unexposed groups. Despite the lack of randomization, this type of studies are designed to mimic randomized controlled trials with respect to protecting against confounding bias that can threaten the internal validity

of a study.[140] Quasi-experimental designs based on family samples or measurements repeatedly taken from the same individual are genetically informative designs that can help control for unmeasured confounders such as genetic background.

5.1.2.1 Sibling comparisons

Sibling comparisons are widely used family-based quasi-experimental designs, especially in studies of associations between prenatal exposures and later health outcomes. In traditional epidemiologic research, unrelated individuals with different exposure levels are compared for prevalence or incidence of outcomes and confounding adjustment solely relies on researchers being able to identify confounders and reliably measure them. Sibling comparisons estimate exposure-outcome associations among differently exposed siblings within a nuclear family and, by design features, automatically account for familial confounding, i.e., confounding bias arising from all familial factors (both genetic and environmental factors) shared by siblings.[116,141] In fact, these familial factors may not be objectively shared, but need to have identical effective influence on siblings.[142] If the exposure under study is a parental trait, full sibling comparisons can preclude confounding due to passive gene-environment correlations (rGEs), because parents pass down their alleles randomly to their offspring during meiosis and the version of alleles carried by each offspring is thereby exposure-independent.[55] When prenatal traits serve as exposures, confounding due to active and evocative rGEs can be ruled out as well because offspring cannot reversely select themselves into or elicit exposures preceding their own births.[55] In addition, sibling comparisons can handle precisely measured covariates by multiple regression model adjustment in the same way as traditional epidemiologic research. Accordingly, significant within-family associations between prenatal exposure and later health outcome cannot be attributed to familial factors that make siblings similar and therefore may serve as more rigorous evidence for potential causal effects; non-significant within-family associations, on the other hand, may indicate, among other things, that familial confounding is likely to play a role in population-level associations.[141] In Study III, sibling-comparison was used to investigate the role of familial confounding in the association between high maternal pre-pregnancy BMI, a prenatal maternal trait, and offspring ADHD.

5.1.2.2 Within-individual comparisons

Within-individual comparisons represent a type of underused genetically informative designs, especially in pharmacoepidemiologic research. By using each study participant as his or her own control, within-individual comparisons automatically control for all time-constant factors (e.g., genetic background and baseline disease severity) within the same individual during the follow-up. Hence, when RCTs are not feasible for studying rare adverse events in relation to pharmacotherapies, observational studies using within-individual comparisons may serve as attractive alternatives. In addition, within-individual comparisons are appealing because of their potential to minimize bias derived from confounding by indication, i.e., indication for treatment is associated with the risk of outcome under study. In Study IV, we examined whether the use of ADHD medication was associated with an increased risk of

concomitant suicidal behavior. The study used within-individual comparisons to control for confounding due to the association between ADHD per se and suicidal behavior. This is reasonable because the association may to some extent be explained by (1) common genetic etiology between ADHD and suicidal behavior, (2) baseline severity of ADHD, and (3) other baseline comorbid psychiatric conditions; all these factors are relatively stable within the same individual.

5.2 COHORT DESIGNS

5.2.1 Standard cohort studies

Cohort studies are a form of longitudinal studies. In a cohort study, an outcome-free study population is first identified. The participants are categorized according to their exposure status and subsequently followed over a certain length of time for the occurrence of an outcome event. If a cohort is a static cohort and all the participants have complete follow-up, we can compare the risk of the outcome between the exposed group and the unexposed group.[143] If a cohort is a dynamic cohort in which not all participants have complete follow-up and each participant contributes a certain length of time at risk, such data on the outcome (event) are called time-to-event data and should be analyzed in the framework of survival analysis. Study III and IV are cohort studies based on prospectively collected data in the Swedish national registers.

5.2.2 Reconstructed cohort studies

In this thesis, the familial aggregation and co-aggregation studies (Study I and II) can be viewed as reconstructed cohort studies, in which index persons defined the exposure (i.e., family history of a condition) and the relatives of the affected and unaffected index persons were "reconstructed" into exposed and unexposed groups. The relatives constituted the study cohort and defined the outcome.[138] In Study I, ADHD status in index person was treated as a time-varying exposure and attained age at first ADHD diagnosis in the relative of the index person was used as a time-to-event outcome. The familial aggregation of ADHD was analyzed in the above-mentioned framework of survival analysis. In Study II, since it is unlikely that a male index person's BMI would influence his relatives' awareness or concern about ADHD, we treated ADHD in the relatives of the index persons as a binary outcome (presence/absence) and estimated the strength of the familial co-aggregation of ADHD and overweight/obesity using logistic regression models.

5.3 STATISTICAL METHODS

5.3.1 Cox proportional hazards models

Cox proportional hazards models are widely applied statistical models for analyzing time-to-event data. When using Cox models, researchers are interested in estimating hazard ratio (HR) as a function of all independent variables. The models assume that hazards for exposed group and unexposed group are proportional throughout follow-up, without

making additional assumption about the shape of baseline hazards.[144] Large register-based data often have adequate power to detect even a slight violation of the proportionality. Under such circumstances, graphical assessment of the violations of the proportional hazards assumption should be carried out. If the assumption is found to be severely violated, stratified analyses should be performed accordingly. In Cox models, HRs are automatically adjusted for the underlying time scale such as attained age.

5.3.2 Logistic regression models

Logistic regression models are commonly employed in epidemiological research when outcome variable is binary. The models assume that the log-odds of a binary outcome variable has a linear relationship with a set of covariates including exposure. The logistic regression coefficient of the exposure variable is interpreted as the change in the log-odds of the outcome for every unit change in the exposure while holding all other covariates constant. By taking the exponential of the regression coefficient, an OR (i.e., the odds of being exposed given the presence of the outcome compared with the odds of being exposed given the absence of the outcome) can be obtained.

5.3.3 Clustered data

Studies using large register-based data increase the potential of data clustering. Examples of clustered data are measurements taken from the same family or repeatedly taken from the same individual. Most statistical models assume independence between observations, but data within a cluster tend to be positively correlated. Failure to address the non-independence of clustered data would lead to underestimation of standard errors and p-values. Thus, special attention should be given to analyses of clustered data to avoid misinterpretations due to incorrect precisions. In between-cluster analyses, a sandwich formula[145] can be used to correct standard errors. The formula accounts for model misspecifications due to data clustering and generates so-called robust standard errors. In this thesis, robust standard errors were estimated in between-cluster analyses in all four studies.

In within-cluster analyses (e.g. sibling-comparison analyses and within-individual analyses), clustered data are not threats to the validity of a study; rather, they can be utilized to control for unmeasured cluster-constant confounding. In this thesis, we used stratified Cox proportional hazards models, which incorporate fixed effects models into Cox proportional hazards models, to investigate exposure-outcome associations in Study III and IV. A stratified Cox model can be viewed as an ordinary Cox model conditioning on each cluster. While ordinary Cox models estimate exposure-outcome associations between unrelated individuals, stratified Cox models estimate exposure-outcome associations within each cluster, assuming with-cluster association is constant across all clusters.

5.4 METHODS BY STUDY

5.4.1 Overview

Study	Participants	Measures	Statistical analyses
I	Relative pairs of varying degrees of genetic relatedness (n = 8,263,689) identified from individuals born in Sweden between1985 and 2006 (n = 1,656,943)	Time-varying exposure: ADHD status in index persons Outcome: Time to first ADHD diagnosis in relatives of index persons Covariates: Relative's birth year	Cox proportional hazards models and robust sandwich estimator
II	Male index persons born between1973 and 1992 (n = 472,735) and their male and female siblings (n = 523,237) and cousins (n = 2,138,440)	Exposure: BMI in index persons Outcome: ADHD in index persons, ADHD in relatives Covariates: Index person's birth year and ADHD status	Logistic regression models and robust sandwich estimator
III	Individuals born in Sweden between 1992 and 2000 (n = 673,632)	Exposure: Maternal pre-pregnancy BMI Outcome: Time to first ADHD diagnosis in offspring Covariates: Offspring sex, birth order, birth year, mother's country of birth, highest education, age at delivery, smoking during pregnancy, and cohabitation with child's father at childbirth	Entire cohort: Cox proportional hazards model with robust sandwich estimator Sibling comparison: Stratified Cox proportional hazard model
IV	Patients with ADHD born between 1960 and 1996 (n = 37,936)	Time-varying exposure: Treatment status by ADHD medications Outcome: Time to first suicide-related event Covariates: Sex, age, previous number of treatment switches, and previous number of suicide attempts	Entire cohort: Cox proportional hazards model with robust sandwich estimator Within-individual: Stratified Cox proportional hazards model

5.4.2 Study I–IV

Study I

By linking the MBR, the MGR, and the STR, we identified all possible relative pairs of monozygotic twins, full siblings, maternal and paternal half siblings, full cousins and half cousins from individuals born alive in Sweden between 1985 and 2006. Full cousins were defined as children of full siblings. Half cousins were defined as children of half siblings. Children of monozygotic twins and individuals who shared both sets of grandparents (i.e., double first cousins) were excluded from the cousin sample because they were genetically equivalent to half siblings. Individuals from single-child nuclear families were also excluded from the cousin sample to ensure that the cousin sample and the sibling sample were comparable. All individuals were followed from their third birthday until first ADHD diagnosis, death, emigration, or December 31, 2009, whichever occurred first.

For descriptive purposes, we plotted cumulative probability of ADHD diagnosis up to 20 years of age in all participants, all siblings, and all cousins, using the Kaplan-Meier method. We then calculated crude and birth year adjusted HRs for ADHD diagnosis in relatives of index persons using Cox proportional hazards models, with ADHD diagnosis in index person as a time-varying exposure and attained age of relative as the underlying time scale. Since each family typically contributed more than one pair of relatives, we estimated robust standard errors to account for the non-independence of the family-clustered data.

To assess the potential importance of shared environmental influences, we tested whether HRs significantly differ between maternal half siblings and paternal half siblings. Maternal half sibling pairs and paternal half-sibling pairs both share 25% of their segregating genes on average, but maternal half-siblings might share more environmental factors compared with paternal half-siblings for two reasons. First, children born to the same mother tend to share more factors related to intrauterine environment.[146] Second children in Sweden continued to live predominantly with their mothers following parental separation during the study period.[147] Consequently, if shared environmental experiences were responsible for some of the association, we would expect higher HR in maternal half siblings than in paternal half siblings.

Furthermore, we carried out several subgroup analyses in full siblings. To examine sex-specific influence on the familial aggregation, we performed analyses stratified on sex combination of index persons and their siblings. When assessing the impact of persistence of ADHD into adulthood, we restricted analyses to sibling pairs born during 1985 – 1991, and further categorized ADHD affected index persons by their age at last diagnosis (<18 / ≥18years).

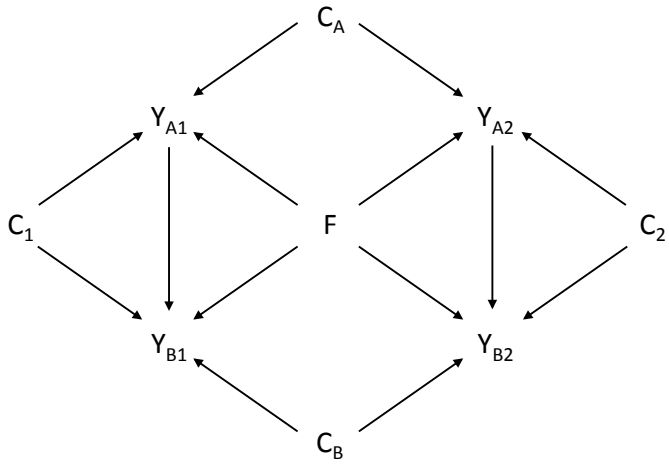
Study II

By linking the MBR, the MSCR, and the MGR, we identified male index persons born alive in Sweden between 1973 and 1992 with information on BMI and their male and female siblings and cousins born in Sweden between 1973 and 2002. Index persons older than 21 years of age at conscription were excluded to diminish age-related variation in BMI, as the vast majority of the index persons were conscripted at age 18-20. Index persons with BMI less than 18.5 were also excluded, because the assumptions used in the study might not apply to the relationship between underweight and ADHD.

Logistic regression models were used to estimate ORs and 95% CIs to assess (1) the association between ADHD and overweight/obesity in index persons and (2) the familial co-aggregation of ADHD and overweight/obesity, i.e., the association between ADHD in relatives of index persons and overweight/obesity in index persons. Robust standard errors were calculated to account for the non-independence of the clustered data.

We used a DAG (Figure 5.4.2.1) to explain the underlying mechanisms of the potential co-aggregation of ADHD and overweight/obesity within families.

Figure 5.4.2.1: Familial co-aggregation of ADHD and overweight/obesity



Y_{A1}: ADHD in index person; Y_{B1}: Overweight/obesity in index person; Y_{A2}: ADHD in index person's relative; Y_{B2}: Overweight/obesity in index person's relative; F: Common familial causes for ADHD and overweight/obesity; C_A: Familial causes for ADHD alone; C_B: Familial causes for overweight/obesity alone; C_1: Common causes for ADHD and overweight/obesity specific for index person; C_2: Common causes for ADHD and overweight/obesity specific for index person's relative.

An important assumption in the DAG is that there is no direct within-individual effect of overweight/obesity on ADHD, i.e., ADHD cannot be caused by overweight/obesity. This

assumption is plausible because ADHD status in the present study was ascertained via clinical diagnosis rather than cross-sectional questionnaires where ADHD-like symptoms may derive from overweight/obesity related conditions such as sleeping disorders.[82] However, the DAG allows for a direct within-individual effect of ADHD on overweight/obesity, i.e., ADHD may cause overweight/obesity.

We used two models to estimate the associations between ADHD in relatives of index persons and overweight/obesity in index persons. Model 1 was used to demonstrate the familial clustering of the two conditions due to any factors shared by family members (C_A and F in this study). This model was not adjusted for ADHD status of index person (Y_{A1}). Therefore, ORs measured the association that can be attributed to the pathway of interest ($Y_{B1} \leftarrow F \rightarrow Y_{A2}$) and an additional pathway mediated by the direct effect of ADHD on obesity ($Y_{B1} \leftarrow Y_{A1} \leftarrow C_A \rightarrow Y_{A2}$).

Model 2 was used to detect the presence of common familial causes for ADHD and overweight/obesity (F). This model was adjusted for ADHD status of index person (Y_{A1}). Such adjustment blocked the path $Y_{B1} \leftarrow Y_{A1} \leftarrow C_A \rightarrow Y_{A2}$ but opened two previously blocked paths, $Y_{B1} \leftarrow C_1 \rightarrow Y_{A1} \leftarrow C_A \rightarrow Y_{A2}$ and $Y_{B1} \leftarrow F \rightarrow Y_{A1} \leftarrow C_A \rightarrow Y_{A2}$.[148] Under realistic scenarios the first path ($Y_{B1} \leftarrow C_1 \rightarrow Y_{A1} \leftarrow C_A \rightarrow Y_{A2}$) is likely to induce an inverse association–component between Y_{B1} and Y_{A2}, because of the adjustment of the collider Y_{A1} and assumption of positive associations represented by all arrows. Thus, if the net association is positive (e.g. ORs > 1), we may conclude that the path $Y_{B1} \leftarrow F \rightarrow Y_{A2}$ and/or the path $Y_{B1} \leftarrow F \rightarrow Y_{A1} \leftarrow C_A \rightarrow Y_{A2}$ is present. As both paths go through F, we may thus conclude that F is present. Note that F may also cause the occurrence of ADHD and overweight/obesity to the same individual. The presence of F indicates an etiological overlap between ADHD and overweight/obesity.

Study III

We identified eligible individuals born in Sweden between 1992 and 2000 from the MBR. We linked the cohort to the MGR to further identify full biological siblings nested in the cohort. All individuals were followed from their third birthday until first ADHD diagnosis, death, emigration, or December 31, 2009, whichever occurred first.

Cox proportional hazards models were used to estimate population-average HRs for time to first-ever ADHD diagnosis and robust standard errors were used to adjust the 95% CIs for the presence of familial clustering. Individuals born to mothers of underweight, overweight, and obesity were compared with individuals born to mothers of normal weight. The models were adjusted for offspring sex, birth order, birth year, mother's country of birth, highest education, age at delivery, smoking during pregnancy, and cohabitation with child's father at childbirth.

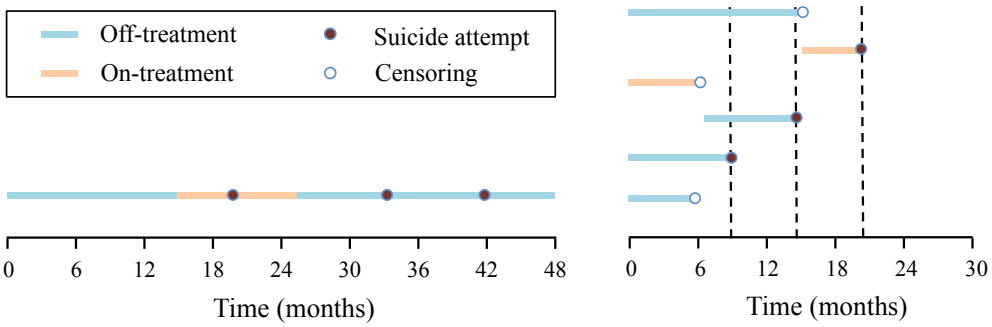
Stratified Cox proportional hazards models were used for sibling comparisons, with each set of full siblings as a separate stratum. The models were adjusted for the aforementioned covariates, except for mother's country of birth and highest education, because these two covariates were constant within families and thus already adjusted by the sibling-comparison design. We also examined the studied association when (1) pooling together the subgroups exposed to maternal pre-pregnancy overweight and obesity and (2) using continuous BMI as the exposure.

We performed additional analyses stratified on the pattern of between-pregnancy variation in BMI (normal weight – normal weight, normal weight – overweight/obesity, overweight/obesity – normal weight, and overweight/obesity – overweight/obesity) in a subsample consisting of first- and second-born siblings from each family. Continuous BMI was used for these analyses to boost statistical power.

Study IV

We identified individuals born between 1960 and 1996 with at least one ADHD diagnosis from the NPR. We linked the cohort to the PDR to retrieve information on dispensed ADHD medications and dispensing dates for all participants.

Figure 5.4.2.2: Data preparation for an ADHD patient with repeated suicide attempts during the follow-up



Treatment status by ADHD medications was treated as a time-varying exposure. We divided the follow-up time into consecutive periods for each participant (Figure 5.4.2.2). A new period started after a treatment switch (i.e., from on-treatment to off-treatment or vice versa) or a suicide attempt. A period following a treatment switch was left truncated at the time of the switch; a period following a suicide attempt started at baseline (i.e., a patient continued contributing time of follow-up after a suicide attempt). A period ending with a treatment switch, emigration, or death due to other causes than suicide was considered censored. All participants were followed from January 1, 2006 to December 31, 2009 for any suicide-related events, death, or emigration.

We performed both between-individual analyses and within-individual analyses to examine the association between the use of ADHD medication and concomitant suicidal behavior. Cox proportional hazards models were used for between-individual analyses, with robust standard errors accounting for correlated data from the same individual. These models were adjusted for sex and categorical age per year. Stratified Cox proportional hazards models were used for within-individual analyses, with each individual as a separate stratum. These models were adjusted for categorical age in years, previous number of treatment switches, and previous number of suicide attempts.

Several sensitivity analyses were conducted to test the robustness of the results. Specifically, two alternative exposure definitions were used to examine the potential influence of exposure time misclassification. First, we set the end of each treatment period to be 30 days after the last drug dispensation. Second, we defined treatment status by using a three-month cut-off (i.e., on-treatment period was defined as a sequence of drug dispensations without discontinuation of more than 92 days). Furthermore, we repeated between-individual and within-individual analyses in subgroups: stimulant users, non-stimulant/mixed users, a younger cohort born during 1982 – 1996 (baseline age 10 – 24 years), and an older cohort born during 1960 – 1981 (baseline age 25 – 46 years).

6 ETHICAL CONSIDERATIONS

Ethical considerations in research are mainly dealing with finding a sensible balance between the intended public good and the level of personal invasion. According to current Swedish regulation, informed consent is not required when register data are used for the purpose of research. However, register-based research should be performed in the corresponding data protection framework to ensure personal integrity of research participants. In this thesis, data on demographic characteristics and healthcare were routinely collected by the Swedish NBHW and stored in the national registers. Such register data were anonymized before being used for research. Specifically, Statistics Sweden, an independent government agency, was responsible for substituting PINs with random identifiers. The code used for linking the PINs and the random identifiers was deleted immediately afterwards to ensure no integrity-sensitive information could be traced back to individual participants. In this thesis, all studies have been approved by the ethics committee at Karolinska Institutet, Stockholm.

Good research includes accurately and effectively communicating research findings to the public. General readers do not gain scientific knowledge through peer-reviewed journals or textbooks as researchers do. It is preferable that researchers provide non-technical interpretations of research findings along with clear descriptions of study participants and the generalizability of the findings through mass media such as television, radio, newspapers, and the Internet. Some findings in this thesis emphasized the importance of genetic origin in the development of ADHD and its associated adverse health outcomes. These findings might be mistakenly interpreted as showing that individuals from families at risk are deemed to suffer from mental health issues. Such simplistic interpretation fails to see that the word "predisposition" per se denotes a tendency rather than a destiny and implies the role of a complex interplay between multiple genetic and non-genetic factors (e.g. environmental factors and life experiences) in the development of a complex disorder. Although the role of environmental factors have not been the core focus of this thesis, the results presented herein do not imply that environmental factors are not of importance.

Despite the valuable implication in scientific literature, negative research findings are often under-reported, which constitutes a major publication bias. In this thesis, we have reported null findings in Study III and IV. We believe the null findings may be beneficial to the planning of future research and implicated in health promotion and disease prevention. However, statistically non-significant results suggest a lack of evidence for rejecting the null hypothesis and should not be viewed as evidence for no associations or no increased risks. This has been carefully discussed in Study III and IV via explicitly pointing out that an increased risk should not be fully ruled out given the upper limit of the confidence interval.

7 RESULTS

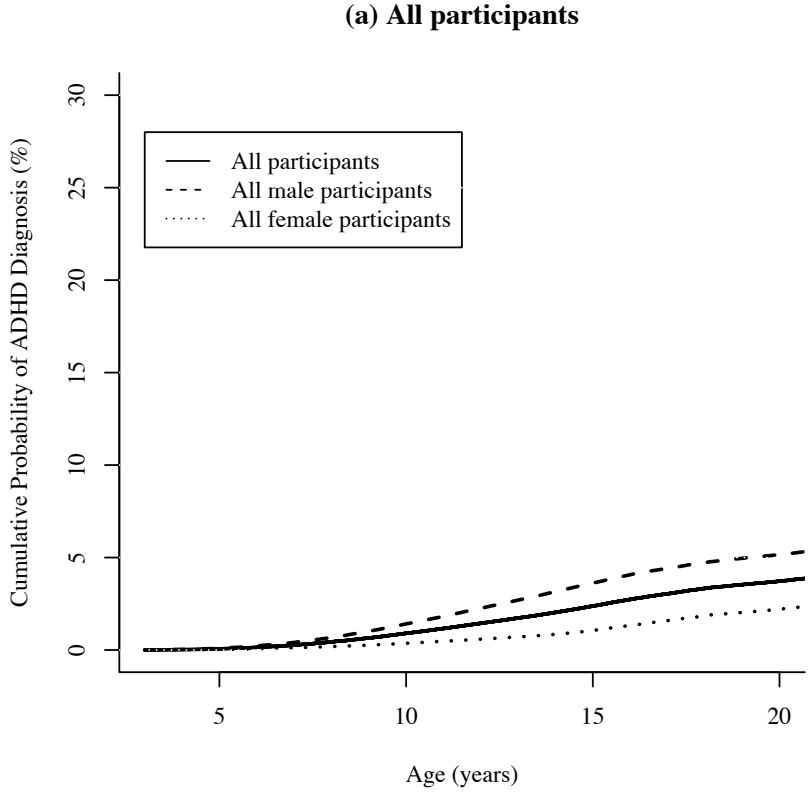
7.1 FAMILIAL AGGREGATION OF ADHD (STUDY I)

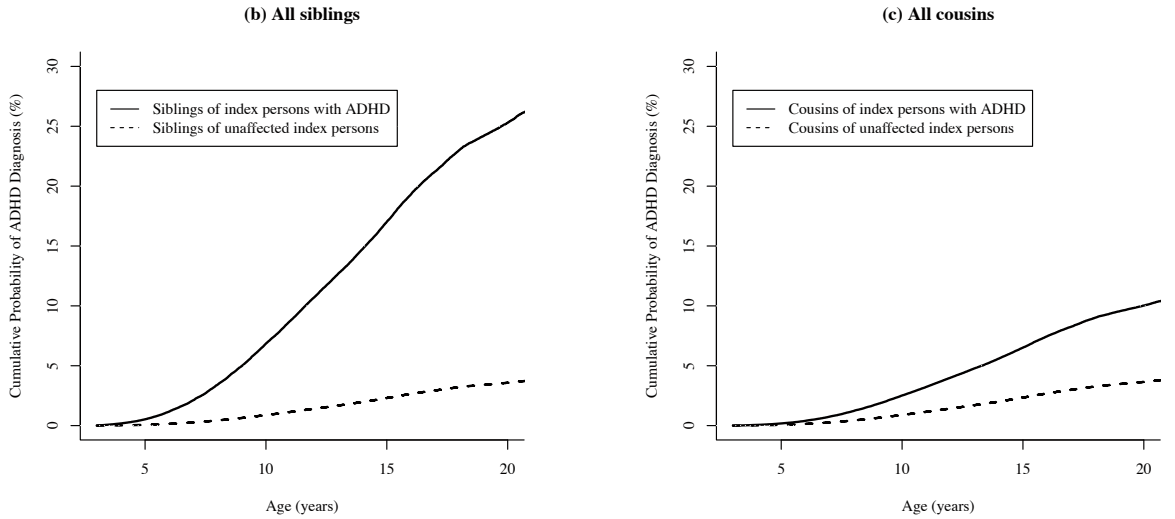
In this study, we quantified the familial aggregation of ADHD in a Swedish family sample consisting of 8,618 monozygotic twin pairs, 26,458 dizygotic twin pairs, 2,030,117 full sibling pairs, 315,267 maternal half sibling pairs, 312,593 paternal half sibling pairs, 4,612,179 full cousin pairs, and 958,457 half cousin pairs constructed from 1,656,943 unique individuals born between 1985 and 2006. During the follow-up, 31,865 individuals received ADHD diagnosis (male to female ratio 3.7).

Cumulative probability of ADHD diagnosis

The cumulative probability of ADHD diagnosis at age 20 for all participants was 3.7% (Figure 7.1 a), which was comparable to the cumulative probabilities for siblings and cousins of unaffected index persons (both around 3.6%). The cumulative probability of ADHD diagnosis at age 20 was 25.3% for siblings (Figure 7.1 b) and 10.0% for cousins (Figure 7.1 c) of ADHD affected index persons.

Figure 7.1: Cumulative probability of ADHD diagnosis in all participants, all siblings, and all cousins

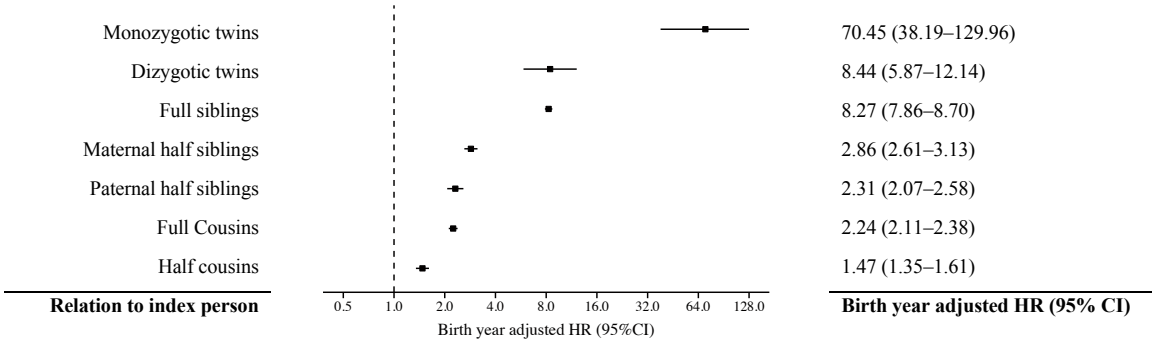




Familial aggregation of ADHD measured by HRs

ADHD aggregated in families, with full siblings of ADHD affected index persons having a more than 8-fold increase in the rate of ADHD diagnosis compared with full siblings of unaffected index persons. The strength of the familial aggregation decreased with decreasing genetic relatedness between family members (Figure 7.2). Maternal half siblings had significantly higher HR compared with paternal half siblings (p = 0.0035).

Figure 7.2: Familial aggregation of ADHD among relatives of varying degrees of genetic relatedness



In full siblings, males had generally higher rate of ADHD than females. HRs did not significantly differ by index person's sex either in male siblings (p = 0.0636) or in female siblings (p = 0.3400) (Table 7.1). Siblings of index persons with ADHD diagnosis persisting into adulthood had increased rate of ADHD compared with siblings of index persons with ADHD diagnosis but only prior to adulthood (Table 7.1).

Table 7.1: Aggregation of ADHD in full sibling pairs by index person's sex and persistence of ADHD into adulthood

		Rate of ADHD per 100 000 person-years		
Full siblings		**Exposed**	**Unexposed**	**HR (95% CI)**
Sex combination[a]				
Sibling	Index person			
Male	Male	2468	207	7.61 (7.07 – 8.19)
Male	Female	2974	219	8.57 (7.61 – 9.65)
Female	Male	1373	75	10.05 (9.15 – 11.03)
Female	Female	1709	81	10.85 (9.30 – 12.66)
Index person's age at last ADHD diagnosis[b]				
< 18 years		668	87	4.68 (3.83 – 5.72)
≥ 18 years		1724	87	11.49 (9.97 – 13.25)

[a]Adjusted for birth year

[b]Presence of diagnosis at age 18 or older was used as a proxy for persistence of ADHD into adulthood; the analyses were performed in full siblings born during 1985 – 1991

7.2 FAMILIAL CO-AGGREGATION OF ADHD AND HIGH BMI (STUDY II)

Study II aimed to quantify the familial co-aggregation of ADHD and overweight/obesity in male index persons and their siblings and cousins, followed by testing the hypothesis that ADHD and overweight/obesity share etiological factors. A total of 523,237 siblings and 2,138,440 cousins for 473,735 male index persons were identified. Among index persons, 384 525 (81.3%) had normal body weight, 68,906 (14.6%) were overweight, and 19,304 (4.1%) had obesity.

Table 7.2: Familial co-aggregation of ADHD and overweight/obesity

	OR (95% CI)	
	Overweight	**Obesity**
Siblings		
Model 1	1.14 (1.05 – 1.24)	1.43 (1.24 – 1.62)
Model 2	1.13 (1.04 – 1.22)	1.38 (1.21 – 1.57)
Cousins		
Model 1	1.11 (1.07 – 1.15)	1.30 (1.22 – 1.37)
Model 2	1.11 (1.07 – 1.15)	1.29 (1.22 – 1.36)

Model 1: adjusted for birth year of index persons

Model 2: adjusted for birth year and ADHD status of index person

Analyses in index persons

Among male index persons, ADHD was positively associated with overweight (OR = 1.31, CI = 1.19 – 1.44) and obesity (OR = 2.00, CI = 1.74 – 2.30). The results were adjusted for birth year.

Analyses in siblings and cousins

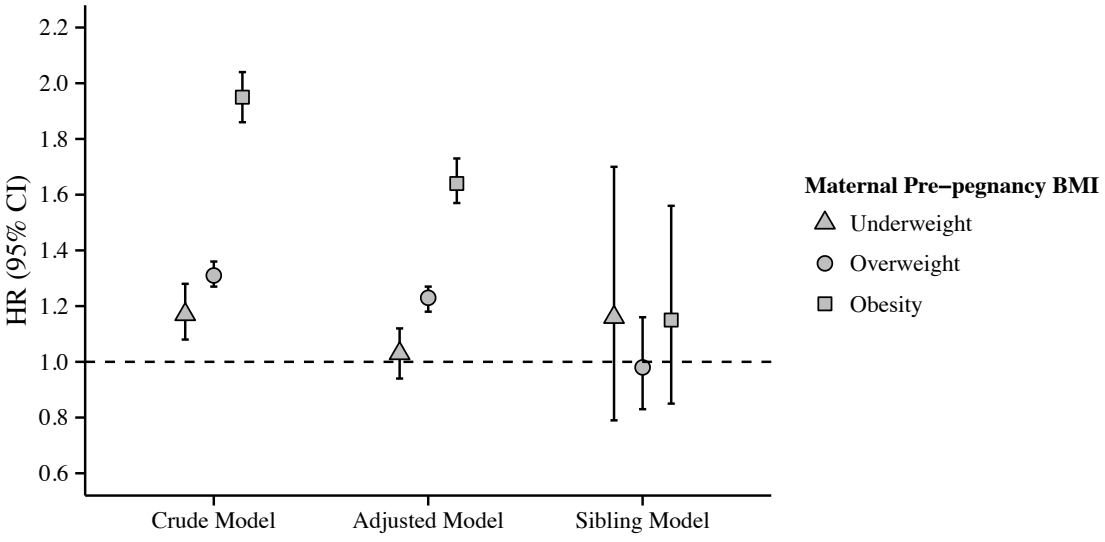
Before adjusting for ADHD status of index person (Sibling Model 1 in Table 7.2), ADHD in siblings of index persons was significantly associated with overweight/obesity in index persons. After adjusting for ADHD status of index person (Sibling Model 2 in Table 7.2), the associations remained significant. Similar results were also observed among index persons and their cousins.

7.3 MATERNAL PRE-PREGNANCY BMI AND OFFSPRING ADHD (STUDY III)

In Study III, we examined whether the association between maternal pre-pregnancy BMI and offspring ADHD was consistent with a causal hypothesis or better explained by familial confounding. The study cohort was composed of 673,632 eligible individuals, including 272,790 full biological siblings nested within 130,060 families.

Crude models showed that maternal pre-pregnancy underweight, overweight, and obesity were all associated with increased risk of offspring ADHD in the entire cohort. After adjusting for measured covariates, the associations for overweight and obesity were slightly attenuated but remained, whereas the association for underweight completely disappeared. Sibling comparisons demonstrated that the observed associations in the entire cohort were largely attenuated to null after adjusting for familial factors shared by siblings (Figure 7.3).

Figure 7.3: Associations between maternal pre-pregnancy BMI and offspring ADHD



Adjusted model: Adjusted for offspring sex, birth order, year of birth, mother's country of birth, highest maternal education, maternal age at delivery, smoking during pregnancy, and cohabitation with child's father at childbirth.

Sibling model: Adjusted for offspring sex, birth order, year of birth, maternal age at delivery, smoking during pregnancy, and cohabitation with child's father at childbirth.

The null effect persisted in the sibling comparison when subgroups of overweight and obesity were pooled together (HR = 0.98; 95% CI = 0.83 – 1.17) and when continuous BMI was used as exposure ($HR_{continuous\ BMI}$ = 0.99; 95%CI = 0.96 – 1.02).

Among first- and second-born siblings, analyses stratified on pattern of maternal variation in BMI between pregnancies revealed that no significant increase in continuous BMI associated with offspring ADHD (Table 7.3).

Table 7.3: Associations between maternal pre-pregnancy BMI and offspring ADHD by pattern of variation in BMI between pregnancies

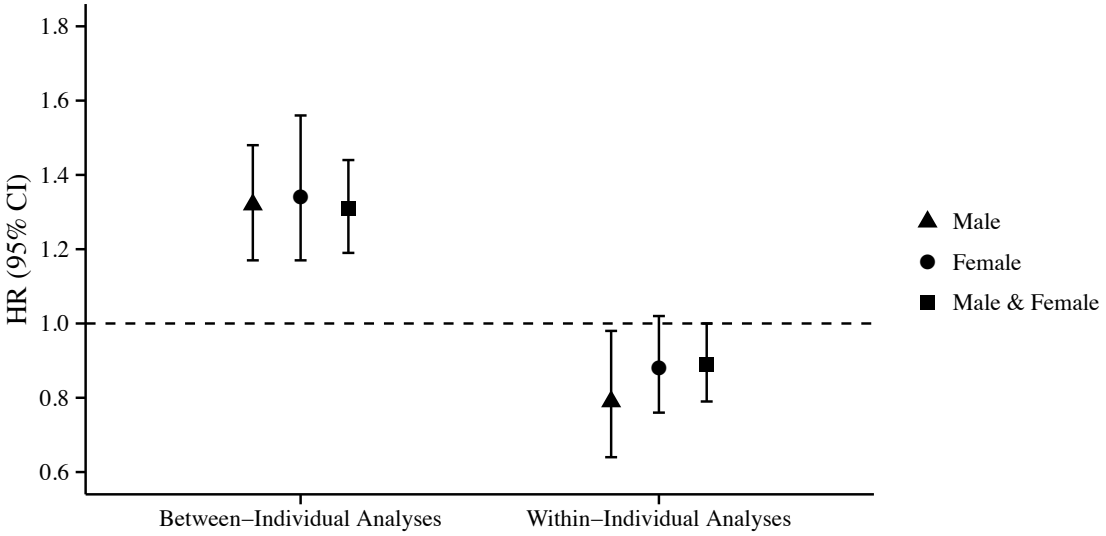
Variation in BMI		HR (95%CI)	
First pregnancy	**Second pregnancy**	**Entire cohort[a]**	**Sibling comparison[a]**
Normal weight	Normal weight	1.02 (1.00 – 1.05)	1.05 (0.97 – 1.13)
Normal weight	Overweight/Obesity	1.05 (1.01 – 1.08)	1.00 (0.93 – 1.08)
Overweight/Obesity	Normal weight	1.01 (0.95 – 1.07)	0.85 (0.67 – 1.08)
Overweight/Obesity	Overweight/Obesity	1.06 (1.04 – 1.07)	0.97 (0.93 – 1.02)

[a]Adjusted for offspring sex and birth order; BMI was analyzed as a continuous variable

7.4 ADHD MEDICATION AND SUICIDAL BEHAVIOR (STUDY IV)

Study IV aimed to investigate whether the use of ADHD medication was associated with an increased risk of concomitant suicidal behaviour using within-individual comparisons to control for time-constant factors within the same individual. A total of 7,019 suicide-related events occurred to 37,936 patients with ADHD during 150,721 person years of follow-up.

Figure 7.4: Association between the use of ADHD medication and concomitant suicidal behavior during 2006-2009



Between-individual analyses were adjusted for categorical age;

Within-individual analyses were adjusted for categorical age, previous number of treatment switches, and previous number of suicide attempts

Between-individual analyses showed that the rate of suicide-related events was 30% higher during on-treatment periods compared to off-treatment periods. HRs among males and females were similar in magnitude (Figure 7.4).

In contrast, within-individual analyses demonstrated no evidence for an increased rate of suicide-related events associated with the use of ADHD medication; in fact, the results indicated a 10% reduction in the rate of suicide-related events during on-treatment periods relative to the rate during off-treatment periods (Figure 7.4).

When alternative definitions of on- and off-treatment periods were used, both between- and within-individual comparisons yielded similar results to the main analyses (Table 7.4). In other sensitivity analyses, between-individual comparisons showed that the use of ADHD medication was associated with an increased rate of suicide-related events in all subgroups except stimulant users. When on- and off-treatment periods were compared within the same individual, no increased rate was observed in any subgroup and a 19% decrease in the rate of suicide-related events appeared to be associated with the use of medication among stimulant users (Table 7.4).

Table 7.4: Sensitivity analyses for the association between the use of ADHD medication and the rate of suicide-related events in subgroups

	HR (95% CI)	
Sensitivity analyses	**Between-individual[a]**	**Within-individual[b]**
Exposure definition		
On-treatment period ended 30 days after last prescription	1.39 (1.27 – 1.53)	0.96 (0.86 – 1.08)
Treatment status defined by 3 month cut-off	1.40 (1.26 – 1.56)	0.90 (0.80 – 1.01)
Subgroup		
Stimulant users	1.02 (0.90 – 1.16)	0.81 (0.70 – 0.94)
Non-stimulant/mixed users	1.49 (1.27 – 1.76)	0.96 (0.77 – 1.20)
Stimulant on-treatment vs. off-treatment	1.45 (1.22 – 1.73)	0.93 (0.72 – 1.20)
Non-stimulant on-treatment vs. off-treatment	1.48 (1.17 – 1.88)	0.96 (0.72 – 1.30)
Younger cohort born during 1982 – 1996	1.17 (1.05 – 1.31)	0.92 (0.79 – 1.07)
Older cohort born during 1960 – 1981	1.39 (1.20 – 1.61)	0.82 (0.68 – 0.99)

[a]Adjusted for sex and categorical age

[b]Adjusted for categorical age, previous number of treatment switches, and previous number of suicide attempts

8 DISCUSSION

8.1 MAIN FINDINGS AND INTERPRETATIONS

8.1.1 ADHD aggregates in families

In Study I, we demonstrated the familial aggregation of ADHD in to date the largest Swedish family sample. The observed familial aggregation of ADHD increased with increasing genetic relatedness between family members. Full siblings of index persons with ADHD had a more than eight-fold increased rate of ADHD compared with full siblings of index persons without ADHD. The findings are consistent with previously reported relative recurrence risk of ADHD in siblings.[149]

In line with previous quantitative and molecular genetic studies,[150,151] genetic origins may explain the pattern of familial aggregation of ADHD among close and distant relatives. In contrast to previous twin research,[152] our finding that maternal half siblings had more pronounced HR compared with paternal half siblings suggests that part of the familial aggregation of ADHD is due to shared environmental influence. Half siblings share on average 25% of their segregating genes, but compared with paternal half siblings, maternal half siblings tend to share more environmental factors related to pregnancy, including intrauterine environment and perinatal conditions.[146] In addition, paternal discrepancy may account for the slightly diminished HR in paternal half siblings. Paternal discrepancy occurs when the identified biological father of a child is in fact not the true biological father. One review based on 17 studies, including a Swedish study performed in1980, has reported a median proportion of paternal discrepancy of 3.7%.[153] In the presence of paternal discrepancy, the assumed paternal half siblings are genetically equivalent to unrelated individuals. Furthermore, we observed higher than average baseline rate of ADHD diagnosis among half siblings, which suggests that having a half sibling might serve as an indicator of encountering more negative life events,[154] or that interpersonal traits correlated with the genetic liability to ADHD are over-represented among the parents of half siblings.

As expected,[155] more males than females received ADHD diagnosis, giving a male to female ratio of 3.7. Despite the difference in the baseline rate of ADHD between males and females, HR did not significantly differ by the sex of the index persons; thus, it is reasonable to assume similar etiology of ADHD for males and females. Consistent with prior studies,[156,157] we observed that full siblings of index persons with ADHD diagnosis at age 18 or older were at higher risk of ADHD than full sibling of index persons with ADHD diagnosis but only before age 18. The results indicate that persistence of ADHD into adulthood is associated with a stronger genetic load compared to remission of ADHD prior to adulthood,[40,158] which makes family members of individuals with persistent ADHD an important target group for diagnostic screening.

8.1.2 ADHD and overweight/obesity share etiological factors

In Study II, we found that ADHD and overweight/obesity were associated within male index persons and co-aggregated within families. Males with ADHD were found to have two-fold increased odds of obesity compared to males without ADHD. The OR was higher than previously estimated according to a recent meta-analysis.[79] The familial co-aggregation of ADHD and overweight/obesity can be jointly explained by (1) the familial aggregation of ADHD, along with the direct within-individual effect of ADHD on overweight/obesity, and (2) common familial causes for ADHD and overweight/obesity. After adjusting for the ADHD status of the index person, the positive associations between overweight/obesity in index persons and ADHD in relatives of index persons remained significant, suggesting that familial causes for ADHD and overweight/obesity may lead to the familial co-aggregation of the two conditions even in the absence of a direct within-individual effect of ADHD on overweight/obesity. The association between ADHD and overweight/obesity within index persons can thereby be, at least in part, attributed to an etiological overlap between ADHD and overweight/obesity.

In Study III, analyses in the entire cohort showed that maternal pre-pregnancy overweight and obesity were both associated with increased risk of offspring ADHD even after adjusting for several measured covariates such as birth order and maternal age at delivery. The magnitude of the observed population-level associations was similar to previously estimated.[159,160] However, the associations lacked statistical significance in sibling comparisons when all factors shared by siblings were accounted for by the study design. Taken together, the causality of the associations under study is not evident; the findings indicate substantial influence of familial confounding on the population-level associations between high maternal pre-pregnancy BMI and offspring ADHD. Familial confounding in this study, by definition, arises from common familial causes for high maternal pre-pregnancy BMI and offspring ADHD. Another way of looking at the familial confounding is that it causes the familial co-aggregation of high BMI and ADHD in mothers and their offspring. Hence, the findings from Study II and III essentially point to the same conclusion that ADHD and overweight/obesity share etiological factors. The study also highlighted that confounding adjustment solely relying on measured covariates is unlikely to be sufficient in epidemiologic research. This reiterates that genetically informative designs such as sibling comparisons, if used wisely, may provide more rigorous tests of competing hypotheses and help make less biased causal inferences in observational studies.[141]

Previous research on ADHD and overweight/obesity has predominantly focused on estimating the association between the two conditions,[79] with the underlying mechanisms of the association being largely unknown. Despite the possibility of ADHD causing obesity through abnormal eating behavior in response to deficient inhibitory control, poor planning, and restlessness,[80] findings from Study II and III suggested that an etiological overlap between ADHD and overweight/obesity may also account for the co-occurrence of the two conditions to the same individual. Study II and Study III to some extent complement each other with regards to sex differences: BMI in male index persons were used in Study II, while

BMI in female index persons were used in Study III. Thus, the familial co-aggregation of BMI and ADHD in both males and females has been investigated. Previous literature has linked the common neurobiological dysfunctions of ADHD and obesity to reward deficiency due to changes in mesolimbic and mesocortical dopamine pathways.[161,162] Such reward deficiency might act as a common distal causal component leading to the development of ADHD and obesity via different biological mechanisms. In addition, mutations in the melanocortin 4 receptor gene and brain-derived neurotrophic factor gene variants have showed possible associations with both ADHD and obesity, even though the evidence is inconsistent.[85,163,164] A recent meta-analysis based on more than 300,000 individuals identified 97 genome-wide significant loci accounting for up to 2.7% of BMI variation.[84] Similar level of progress in molecular genetic research on ADHD has not yet been achieved.[43] More research is needed to determine the degree to which the association between ADHD and overweight/obesity might be driven by their etiological overlap. Apart from family-wide environmental risk, future genetic studies seeking for common pleiotropic genetic variants regulating both ADHD and BMI are warranted and may lead towards further exploration of novel effective treatments for both ADHD and overweight/obesity.

8.1.3 No evidence for an increased risk of concomitant suicidal behavior associated with the use of ADHD medication

In study IV, when comparing different individuals over on-treatment periods and off-treatment periods (between-individual analyses), we observed a 30% increase in the rate of suicide-related events associated with the use of ADHD medication. The association disappeared in within-individual analyses in which each individual was compared with him or herself. If anything, the results pointed to a potential protective effect of ADHD medication on suicidal behavior, particularly for males and stimulant users.

To the best of our knowledge, this is the first nationwide longitudinal study investigating ADHD medication in relation to suicidal behavior. In within-individual analyses, we accounted for both measured time-varying confounding factors, including age, previous number of suicide attempts, and previous number of treatment switches, and unmeasured time-constant factors such as baseline severity of ADHD and its comorbid psychiatric conditions as well as genetic susceptibility to both ADHD and suicidal behavior. The non-significant findings from the within-individual comparisons suggest that the increased rate of suicide-related events associated with the use of medication in between-individual analyses might be due to unmeasured time-constant confounding factors.

In between-individual analyses, off-treatment periods were contributed by medication-free patients and patients with treatment switches, while on-treatment periods were contributed by patients with treatment switches and individuals on medication throughout the follow-up. Compared to patients with mild ADHD, patients suffering from more severe forms of ADHD and other comorbidities such as mood disorder and substance abuse disorder are more likely to be treated by medication and more inclined to commit suicide or suicide attempt. In other words, the significant associations observed in between-individual analyses were possibly

confounded by increased severity of ADHD and high rate of comorbid conditions, a phenomenon commonly referred to as confounding by indication. The within-individual comparisons to a large extent ruled out bias due to confounding by indication, because the severity of ADHD and its comorbid conditions within the same individual was assumed to be relatively stable over time.

There are several possible explanations for the decreased rate of suicide-related events associated with the use of medication among male patients and stimulant users in within-individual analyses. First, this inverse association may represent a protective effect of ADHD medication on suicidal behavior. Such protective effect is biologically plausible in consideration of the well-documented efficacy of ADHD medication in symptom reduction, including reduction in both attentive and impulsive symptoms. Impulsive behavior is overrepresented by males and impulsivity has once been suggested as a promising intermediate phenotype for molecular genetic research in suicidal behavior.[91] Thus a protective effect of ADHD medication on suicidal behavior might be mediated by the improvement of impulsive symptoms. Nevertheless, the protective effect was observed only among stimulant users but not non-stimulant/mixed users, possibly owing to the fact that stimulant users suffered from less complex ADHD and other psychiatric symptoms compared to non-stimulant/mixed users. Second, the protective effect may also be due to so called confounding by contra-indication, i.e., medications were less likely to be prescribed to suicidal patients. Third, having increased rate of suicidal behavior might indicate disorganized periods featured by poor adherence to medication treatment. Further research is needed to clarify whether stimulants indeed have a protective effect on suicidal behavior and if so, the mediating mechanisms of the protective effect.

The findings from this study may inform clinical and public health decision-making. Moreover, within-individual comparisons deserve more attention in pharmacoepidemiologic research attempting to effectively control for time-constant factors within the same individual so as to minimize bias arising from confounding by indication. Additional research based on different designs and samples is needed to further elucidate the effects of stimulants and non-stimulants on suicidal behavior, including the long-term effects.

8.2 METHODOLOGICAL CONSIDERATIONS

8.2.1 Measurement errors and misclassifications

8.2.1.1 ADHD

The ascertainment of ADHD cases in this thesis was predominantly based on ICD-10 diagnosis given by psychiatric specialists and prescription of medication unique for ADHD treatment. The ICD-10 criteria for HDK are more conservative compared with the DSM-IV criteria for ADHD. In Sweden, pharmacotherapy is mainly preserved to patients with moderate to severe ADHD when non-pharmacological interventions alone have failed. Taken together, ADHD cases in this thesis tend to be individuals affected by more severe forms of

the disorder; thus, false negatives cannot be avoided, whereas bias due to false positives is unlikely. Although in a young cohort of Swedish twins, two approaches for the ascertainment of ADHD cases, diagnosis according to the NPR and assessment based parent-rated symptoms have shown considerable agreement,[165] future validation studies on the ADHD diagnoses in the NPR are needed.

Diagnostic criteria for adult ADHD were not available during the study period. The validity of ADHD diagnosis in adults used to be considered controversial.[166,167] Nevertheless, prior studies have consistently reported that ADHD is a relatively stable condition that persists from childhood into adulthood in a substantial proportion of patients and is associated with impairment in both clinical and psychosocial functioning. The DSM-5 has added examples of symptom presentations in adulthood. In Sweden, the diagnostic assessments cover comprehensive aspects of intellectual impairment, autism, psychiatric and somatic comorbidities, and developmental history. Thus, we believe that ADHD diagnoses in adults in this thesis were valid from the perspective of research.

8.2.1.2 Overweight and obesity

Overweight and obesity were measured by BMI in Study II and III. BMI reflects statistical rather than physiological criteria for overweight and obesity. For instance, BMI might not be an optimal proxy for the synthesis and storage of cholesterol in adipose tissue. Individuals with the same BMI value may differ widely in their body composition. Central abdominal adiposity surrounding the viscera has been associated with increased risk of various adverse health outcomes.[168] In contrast, peripheral adiposity in the gluteofemoral region appears to have protective effect against several chronic diseases.[169] Therefore, BMI-measured overweight/obesity might not reflect the real harmful biological components in the process of cholesterol metabolism.

In sibling-comparison studies, reliably measured exposure is crucial to avoid biased estimation due to random measurement errors. This will be further discussed in Section 8.2.3.

8.2.1.3 Treatment status by medication

The ascertainment of treatment period in Study IV was through a sequence of drug dispensations that might inaccurately reflect the actual consumption of medication by the patients due to non-adherence, i.e., the patients might not actually take the medications as prescribed. This might give rise to exposure time misclassification. The problem here is similar to the potential non-adherence to protocol in RCTs. The analyses in Study IV can be compared to intention-to-trait analyses (i.e., data for each participant were analysed according to the original treatment allocation) in most RCTs, which evaluate what would happen in real clinical settings if a treatment were offered.[120] In Study IV, sensitivity analyses based on alternative definitions of on- and off-treatment periods did not invalidate the results from the main analyses, indicating that exposure time misclassification is unlikely to account for the non-significant results in within-individual analyses.

8.2.1.4 Suicide-related events

Deaths from suicides have been validated via mortality statistics in Sweden,[170,171] whereas suicide attempts retrieved from the NPR might be limited to relatively severe cases that were in need of medical care. Since the NPR might not capture mild forms of suicide attempts, the overall rate of suicide-related events might be underestimated, resulting in misclassification of outcome in Study IV.

8.2.2 Estimates in familial co-aggregation studies

In Study II, we first quantified the familial co-aggregation of ADHD and overweight/obesity in siblings and cousins, and subsequently tested the hypothesis that ADHD and overweight/obesity share etiological factors. In the crude models, ORs were used to describe the strength of the familial co-aggregation of ADHD and overweight/obesity in siblings and cousins. In the adjusted models, ORs significantly greater than 1 indicate an etiological overlap between ADHD and overweight/obesity. However, the magnitude of ORs cannot be directly translated into the extent to which the etiology of ADHD overlaps with the etiology of overweight/obesity, because ORs are relative measurements and the magnitude of ORs depends on the prevalence of each condition under study as well as the amount of familial factors jointly causing the coexistence of the two conditions in families. It is possible that familial co-aggregation studies of two highly prevalent conditions would yield relatively lower ORs compared with similar studies of two rare conditions.

8.2.3 Assumptions in sibling comparison studies

All research designs rely on a set of assumptions to provide valid inferences, with sibling-comparison design being no exception. In Study III, the following assumptions must be met before a valid causal inference can be made.

(1) Exposure and other covariates are reliably measured.
Differentially exposed siblings differ in non-shared causes of exposure, including random measurement errors. Differentially exposed siblings are more vulnerable to misclassification of exposure due to random measurement errors [172] (e.g. maternal BMI value close to the cut-offs used for categorizing continuous BMI into normal weight, overweight, and obesity might give rise to misclassification between these categories in Study III). Such misclassification of exposure in Study III were most likely to be non-differential, which might serve as an alternative explanation for the attenuated associations in sibling-comparison analyses.

(2) There is no sibling carryover or contagion effects.
Sibling carryover or contagion effects occur when the exposure or outcome of one individual affects the exposure or outcome of his or her siblings.[141] To test for carryover effects of maternal pre-pregnancy BMI in the first-born sibling on ADHD in the second-born sibling (i.e., asymmetric effects), we analyzed sibling pairs in subgroups divided by pattern of the variation in maternal BMI between pregnancies.

The stratified Cox regression analyses produced similar results irrespective of whether the exposed sibing was first-born or second-born, indicating that such asymmetric sibling carryover or contagion effects, if present, were of limited importance to the overall sibling-comparison analyses.

(3) Differentially exposed siblings are representative for the general population.
As within-family similariy in exposure increases, the number of differentially exposed siblings may dramatically decrease, which might threat the external validity of sibling-comparison studies.[141] Therefore, it is crutial to check if differentially exposed siblings are representative for all siblings and if siblings differ in relevant aspects from individuals without siblings.[173] In Study III, we analyzed siblings as unrelated individuals and compared the estimate with the population-level estimate. The similarity between the two estimates relieved us of the concern regarding external validity.

When these assumptions are plausible, sibling-comparison studies may help scrutinize the role of unmeasured familial confounding in putative exposure-outcome associations and test competing causal hypotheses, even though they cannot prove causality. Causal inferences can be strengthened through convergent evidence from studies using different designs and samples and suffering from mutually exclusive limitations.

8.2.4 Caveats of fixed effects models

8.2.4.1 Unmeasured cluster-varying confounding

Fixed effects models (stratified Cox proportional hazards models in Study III and IV) cannot handle unmeasured confounding factors that vary within each cluster.[174] In Study III, sibling-comparison analyses cannot control for unmeasured confounding factors that were not shared by siblings. In Study IV, within-individual analyses cannot account for time-varying confounders occurring to each individual, such as sporadic onset of comorbid conditions associated with both medication treatment and suicide-related events. To address this issue, we restricted within-individual analysis in a subgroup of ADHD patients without major comorbid psychiatric conditions (i.e., mood disorder, conduct disorder, substance abuse disorder, and borderline personality disorder) and observed no increased rate of suicide-related events associated with the use of ADHD medication.

8.2.4.2 Non-informative clusters

Fixed effects models cannot generate any effect estimates for variables that have no variation within clusters.[174] In Study III, a family was non-informative (1) there was no within-family variation in any covariate in the model, including maternal pre-pregnancy BMI, or (2) all siblings in the family were free of ADHD. In Study IV, individuals without treatment switch or suicide-related event during the follow-up did not contribute information the within-individual analyses. Non-informative clusters may result in loss of generalizability， as

discussed in Section 8.2.3, and loss of precision, which can be reflected by wider confidence intervals and higher p-values. In Study III, we cannot exclude a causal effect of maternal pre-pregnancy obesity on offspring ADHD given that the upper limit of the 95% CI for the HR was 1.56. Nevertheless, such causal effect, if existing, would not be as large as previously estimated. Likewise, an up to 30% increase in the rate of suicide-related events associated with the use of atomoxetine cannot be ruled out in Study IV, as the upper limit of the 95% CI for the HR was 1.30.

8.2.4.3 Non-collapsibility

When exposure-outcome associations are measured by ORs or HRs, adjusting for covariates that are associated with the outcome but independent of the exposure (i.e., non-confounders) may increase the regression coefficient for the exposure. This property of OR and HR is referred to as non-collapsibility. Fixed effects models automatically adjust for all factors that are constant within clusters regardless of whether they are true confounders; thus the models might be prone to the non-collapsibility effect. Non-collapsibility effect is not a bias, but it means that population-level estimates and cluster-specific estimates are derived from different statistical models and have different interpretations.[175] In addition, non-collapsibility effect cannot alter the sign of the association and therefore is not responsible for the non-significant findings in Study III and IV.

8.2.5 Generalizability

The studies involved in this thesis were based on nationwide Swedish samples with prospectively collected information over the past few decades. Nevertheless, lower incidence rate of ADHD diagnosis, lower average BMI value, and less prescriptions of ADHD medication in Sweden than in the United States indicate that the estimates in this thesis might be more transferable to the population in Sweden and other Nordic countries and less to countries with different social and cultural backgrounds and different levels of ethnic diversity.

In Study II, due to lack of information on BMI for females at age 18-20, it is uncertain whether the findings can be generalized to female index persons and their relatives. However, results from Study III seem to support the findings from Study II, even though mothers and their offspring rather than siblings or cousins were investigated in Study III and maternal BMI was measured at childbearing age. Since BMI for male index persons was measured only once in young adulthood, the generalization of the results to other age groups should be made with caution.

In Study III, we were aware of several threats to the representativeness of the exposure. First, mothers with varying BMI across pregnancies might not be representative for mothers with relatively stable BMI with respect to the biological mechanisms of overweight and obesity. It is possible that mothers who had obesity during two concessive pregnancies have more severe health related problems and their offspring were exposed to more adverse prenatal conditions. Second, mothers who had obesity during one pregnancy and managed to lose

weight during the subsequent pregnancy were likely to have better awareness of their health problems and their offspring might not be exposed to an extreme degree of detrimental prenatal environment. These issues were partly addressed by the same set of sensitivity analyses used for testing sibling carryover and contagion effects. These sensitivity analyses showed limited modifying effect by the pattern of maternal between-pregnancy variation in BMI.

In Study IV, one issue to be aware of is whether individuals who discontinued their medication treatment were representative for individuals who adhered to the treatment throughout the entire follow-up. The data showed that a vast majority of study participants had both on- and off-treatment periods during the follow-up, which provided some evidence for an acceptable generalizability of the within-individual estimates based on individuals with intermittent treatment to the entire cohort of individuals with ADHD.

9 CONCLUSIONS

This thesis has demonstrated how epidemiologic research using genetically informative designs has shifted the field from documenting putative associations to better understanding the underlying mechanisms for these associations. The main insights gained from the four studies are as follows:

ADHD aggregates in families. The familial aggregation increases with increasing genetic relatedness. If family members are affected by ADHD that persists into adulthood, the familial aggregation can be particularly strong, suggesting that such families represent an important target group for diagnostic screening.

ADHD and high BMI co-aggregate in families. Such familial co-aggregation of ADHD and high BMI can be at least in part attributed to common familial causes for the two conditions, indicating an etiological overlap between ADHD and high BMI. The etiological overlap can manifest as familial confounding, which plays a role in the empirical association between high maternal pre-pregnancy overweight/obesity and offspring ADHD at the population level.

The population-level association between the use of ADHD medication and an increased risk of concomitant suicidal behavior is likely to be explained by the association between ADHD per se and suicidal behavior as well as other time-constant factors within the same individual.

10 FUTURE PERSPECTIVES

Although the large familial aggregation study of ADHD in this thesis estimated the family risk of ADHD with relatively greater precision, accurate identification of the sources of the familial factors that cause the aggregation will still rely on quantitative genetic studies. Recently, in a Swedish population-based study of familial risk of autism, the researchers estimated the heritability of autism in an extended sibling sample and reported a heritability of approximately 50%, much lower than previously reported heritability estimates for autism.[176] The heritability of ADHD has not yet been estimated in a similar setting.

Joint use of quantitative measures of ADHD symptoms and clinical diagnosis of ADHD may help further elucidate whether there are sex-specific genetic and environmental influences on ADHD, even though such effects were not evident according to Study I. Longitudinal studies with even longer observation time are needed to confirm the influence of persistence of ADHD into adulthood on the familial aggregation of ADHD. Future family studies also need to estimate the familial risk of ADHD among inter-generation relatives such as parents and their offspring to complete the picture of the familial aggregation of the disorder.

Bivariate quantitative genetic studies can be used to future elucidate the relative contributions of genetic and environmental influences on the covariance of ADHD and high BMI. Publically available summary statistics from the results of previous GWASs of ADHD and obesity can be used to estimate the genetic correlation between ADHD and obesity due to common genetic variants. ADHD and obesity polygenic scores can be generated based on these summary statistics, given genotype data from an independent sample. It would be interesting to look at whether joint use of the polygenic scores of these two traits can improve the performance of risk prediction of ADHD, for example, in the sample of the Child and Adolescent Twin Study in Sweden.[177]

Although our findings did not support that high maternal pre-pregnancy BMI may cause offspring ADHD, this does not contradict obesity prevention among women at childbearing age. Pre-pregnancy obesity and weight gain have been associated with many prenatal and perinatal complications, including infant mortality, which is worth further investigation using elaborated measurements and different designs. In vitro fertilization (IVF) studies that once have helped reveal the contribution of familial confounding in the relationship between smoking during pregnancy and ADHD might also help unravel the nature of the relationship between maternal pre-pregnancy high BMI and offspring ADHD. As stated earlier, no single study can provide a definitive answer and the debate over whether high maternal pre-pregnancy BMI may cause offspring ADHD has just begun and will likely continue.

Due to the scarcity of suicide-related events in children, we failed to estimate the impact of ADHD medication on the risk of suicidal behavior solely in this age group. Future pharmacoepidemiologic research in younger patients, especially during initial phase of treatment and following dosage change, remains critical for ensuring the security of the pharmacotherapy of ADHD.

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