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PARENTAL CHARACTERISTICS AND OFFSPRING INTERNALIZING BEHAVIORS - UNDERSTANDING THE ASSOCIATIONS USING QUANTITATIVE GENETICS

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ABSTRACT

Internalizing problems increase dramatically from childhood to adolescence and account for a large proportion of mental health problems worldwide. During the past decade research has documented a robust association between offspring internalizing problems and different aspects of parental characteristics, such as parenting practices and psychiatric disorders. Findings from twin and family studies have suggested that this association is influenced by both genetic and environmental factors. Nevertheless, prior studies have had limited possibilities to disentangle familial confounding from causal environmental mechanisms. Therefore, we aimed to use different genetically informative designs to explore the direction and etiology of the association between different parental characteristics and offspring internalizing behaviors.

In Paper I, we examined a sample of Swedish twins to understand the direction and etiology of the association between different parenting styles and offspring internalizing behavior from adolescence to early adulthood. We found that daughters internalizing behavior influenced an emotional overinvolved behavior from their parents. Twin analyses indicated that this association was mediated by genetic factors.

In Paper II, we investigated the impact of offspring death and suicide on psychiatric disorders among their parents in a cohort defined by nationwide registers. Parents exposed to offspring suicide had considerably higher risk for subsequent psychiatric hospitalization. Furthermore, a shared genetic liability for psychiatric disorder seemed important judging from family-based analyses.

In Paper III, we specifically examined the suicide risk among offspring of parents hospitalized for schizophrenia and the mechanisms behind this association. We observed a doubled risk of suicidal behavior in offspring. Cousin comparisons suggested that environmental factors play an important role in this association.

In Paper IV, we explored if ADHD and suicidal behavior shared genetic and environmental factors. We found an increased risk of both completed and attempted suicide among relatives of individuals with ADHD. The pattern of familial aggregation indicated genetic influences for this association.

In conclusion, genetic and environmental factors contributed to the associations between parental characteristics and offspring internalizing behavior. Internalizing behaviors in offspring predicted both parenting and psychiatric disorders through genetic mechanisms. However, we could also show that specific parental psychiatric disorders predicted offspring internalizing behaviors through environmental mechanisms. In addition, we found that genetic factors for internalizing behavior to some extent is shared with genetic factors for ADHD. Future research using other genetically informative designs to control for familial confounding is necessary to provide a clearer understanding of the etiological link between parental characteristics and offspring internalizing behavior.

LIST OF PUBLICATIONS

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

- I. Moberg T, Lichtenstein P, Forsman M, Larsson H.
Internalizing behavior in adolescent girls affects parental emotional overinvolvement: A cross-lagged twin study.
Behavior Genetics. 2011; 41: 223-233.
- II. Ljung T, Sandin S, Långström N, Runeson B, Lichtenstein P, Larsson H.
Offspring death and subsequent psychiatric morbidity in bereaved parents: Addressing mechanisms in a total population cohort.
Psychological Medicine. 2013. [Epub ahead of print]
- III. Ljung T, Lichtenstein P, Sandin S, D'Onofrio B, Runeson B, Långström N, Larsson H. Parental schizophrenia and increased offspring suicide risk: exploring the causal hypothesis using cousin comparisons.
Psychological Medicine. 2013; 43(3):581-90.
- IV. 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.
Manuscript

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LIST OF ABBREVIATIONS

ADHD	Attention-deficit hyperactivity disorder
AIC	Akaike's information criterion
ATC	Anatomical Therapeutic Chemical
BIC	Bayesian Information Criterion
CI	Confidence Interval
CFI	Camberwell Family Interview
DSM	Diagnostic and Statistical Manual of Mental Disorders
DZ	Dizygotic
EE	Expressed emotion
EOI	Emotional overinvolvement
GLM	Generalized Linear Models
ICD	International Classification of Diseases
MGR	Multi Generation Register
MZ	Monozygotic
NPR	National patient register
OR	Odds ratio
rGE	Gene-environment correlation
RR	Relative risk
SEM	Structural equation modeling
SES	Socio-economic status
TCHAD	Twin Study of Child and Adolescent Development

1 INTRODUCTION

Internalizing problems, such as depression and suicidal behavior, increase dramatically from childhood to adolescence, generally persist into adult age, and account for a large proportion of mental health problems worldwide [1, 2]. Moreover, individuals with internalizing behaviors are also at an increased risk of several externalizing behavior problems (i.e. ADHD) [3]. During the past decade, a substantial number of studies have documented a robust association between offspring internalizing problems and different aspects of parental characteristics, such as parenting practices and psychiatric disorders. However, the direction of these associations have been unclear. For example, do parents with a harsh discipline increase the risk of internalizing problems in their offspring? Or is it internalizing behavior among children that evoke a certain behavior from their parents?

Although previous research indicate an association between parental characteristics and offspring internalizing behaviors, the field lacks in knowledge regarding the genetic and environmental mechanisms underlying these associations. In particular, it is not known whether potential risk factors for offspring internalizing problems act through environmental effects (e.g. mentally ill parents impaired care to their children) or if the association is due to a common genetic susceptibility for psychiatric disorders and internalizing behavior. Thus, genetic factors, which may explain individual differences in predisposition to mental health problems, also need to be explored. Therefore, we aimed to use different genetically informative designs to explore the direction and etiology of the association between different parental characteristics and offspring internalizing behaviors.

2 BACKGROUND

2.1 INTERNALIZING BEHAVIOR

2.1.1 Definition

Internalizing behavior refer to a large group of emotional behaviors which are directed towards the self. These types of problems include symptoms related to anxiety and depression as well as withdrawn, phobic and suicidal behaviors. Internalizing behaviors is a construct that has been extensively used in child and adolescent psychiatry although the classification of the term has been debated[4]. The internalizing spectrum includes several psychiatric symptoms but has primarily been defined by the two most common disorders, depression and anxiety [5]. These internalizing symptoms, especially depressed mood, have also been found to be particularly common among children with suicidal behavior [6], which suggests that suicidal behavior could be included in the internalizing construct. In this thesis, suicidal behavior refers to individuals who have attempted or completed suicide.

There are two commonly used guides to classify psychiatric disorders the Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Classification of Diseases (ICD). Both approaches are used worldwide although the DSM has been frequently used in the United States whereas the ICD more so in the European countries. Another way to classify internalizing behavior problems has been through empirically based assessments, a system developed by Achenbach and colleagues [7, 8]. This assessment has quantified internalizing behaviors into three subscales: anxious/depressed (e.g., “self-conscious or easily embarrassed”), withdrawn/depressed (e.g., “would rather be alone than with others”), and somatic complaints, and has been demonstrated to be directly related to diagnoses from the DSM. Thus, it is possible to identify internalizing behaviors through information from questionnaires completed by different informants as well as through diagnoses set by physicians.

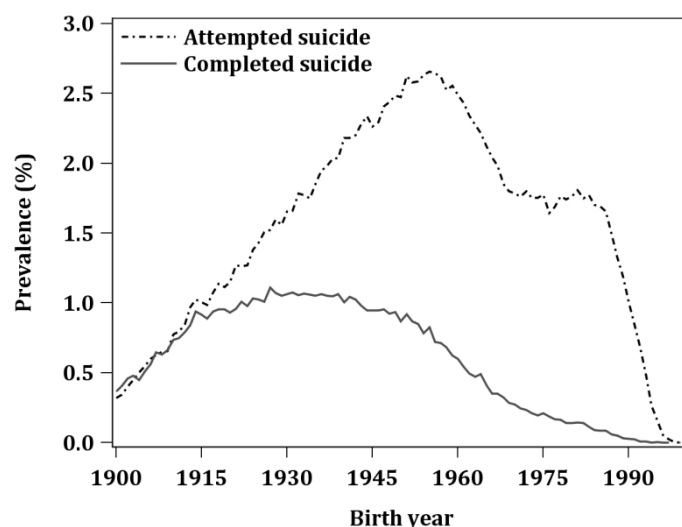


Figure 1. Distribution of suicidal behavior by birth year

2.1.2 Occurrences

Internalizing behaviors account for a large proportion of mental health problems among children around the world and the incidence increase from childhood to adolescence. The prevalence has been assessed in a representative population-based sample, which reported that at least 15% develop an emotional psychiatric disorder by 16 years of age [1]. Specifically, suicidal behavior has become a major public health problem with an estimated prevalence of 4.1% for attempted suicide among adolescents in the US [9]. In Sweden, the annual prevalence of suicidal behavior is around 2% for attempted suicide and approximately 1% for completed suicide (see Figure 1). However, the rates of internalizing disorders are most likely underestimated since internal problems are difficult to communicate, especially for children [10].

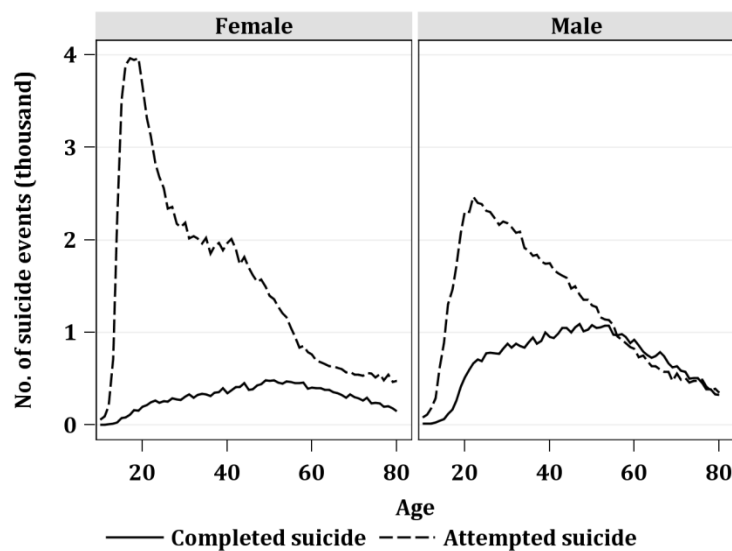


Figure 2. Suicidal events by age and gender (1900-1999)

It is well known that the development of internalizing problems is different for boys and girls. During childhood the levels of internalizing problems show no evident difference between girls and boys, whereas in adolescence girls show a dramatic increase of internalizing behaviors compared to boys [11-13]. Adolescent girls are twice as likely to experience anxiety and depression, a pattern which continues into adulthood [2]. Several studies have further reported that men are more likely to complete suicide than females although females have higher rates of attempted suicide [9]. This same pattern is reflected in Swedish population-based registers (Figure 2) and might be explained by males' use of more lethal methods (such as hanging) compared to females' use of self-poisoning methods [14]. Figure 2 confirms the increasing rates of suicidal behavior from childhood to adulthood among both boys and girls.

The increased risk of internalizing behavior in girls might depend on their social orientation towards relationships, which increase their vulnerability to interpersonal stress and negative relationships [15]. However, there are some

theories that suggest that the increased risk is due to genetic differences between genders [12].

2.1.3 Psychiatric comorbidities

Internalizing disorders during adolescence have been found to co-occur with externalizing behaviors (i.e. ADHD and substance use disorders) [16-19]. A recent finding suggest that more than 40% of adolescents which undergo substance use treatment have established symptoms for both internalizing and externalizing problems [3]. Similarly, the vast majority (90%) of children and adolescents with suicidal behavior have a co-occurring psychiatric disorder [20, 21] or clustering of personality traits such as impulsivity and aggression, which might mediate the familial transmission of suicidal behavior [22-24]. Specifically, suicidal behavior has been associated with psychiatric disorders such as schizophrenia, affective disorders, substance use disorders, ADHD and personality disorder [25-27]. Nevertheless, it is important to note that the majority of individuals with psychiatric disorders do not attempt suicide, which indicates that psychiatric disorders are an important but not sufficient cause of suicidal behavior. Many psychiatric disorders are genetic in origin and the overlap between comorbid disorders might indicate a common genetic vulnerability. Thus, a better understanding of the mechanisms associated with internalizing problems and psychiatric comorbidities are important to find more effective ways of identifying and modifying the risk of internalizing problems.

2.2 PARENTAL CHARACTERISTICS

Parental characteristics have been accepted as important risk factors in the development of internalizing behavior [28-32]. There are two important dimensions of parental characteristics: a wide range of parenting practices (i.e. parental strictness, control, warmth, acceptance, and involvement) as well as different parental psychiatric disorders. Previous studies have found that a cold and controlling manner of parenting contributes to the development of depression in offspring [33]. Likewise, an overprotective parenting style might also increase children's vulnerability to internalizing behaviors [34, 35]. However, establishing causation between parental characteristics and offspring internalizing has been challenging, because children might also evoke a certain behavior from their parents [36].

It has been suggested that the association between parental characteristics and internalizing behavior might be driven by both genetic and environmental effects [37, 38]. For example, parents with psychiatric disorders might provide compromised care to their children, and could also transmit some part of the susceptibility genes to their offspring. Thus, both genetic and environmental factors might influence the association between parental characteristics and offspring internalizing behaviors but the direction and the etiology underlying these associations have not been clarified.

2.3 DIRECTION OF EFFECTS

Most previous research with a focus on the association between different components of parental characteristics and offspring internalizing problems has examined the role of parent-driven effects (i.e. the effect of parents' characteristics on offspring behavior). However, studies have also highlighted child-driven effects as an important risk mechanism [34, 35]. Child-driven effects are present when the child's behavior evokes a parental behavior towards the child. Evidence of child-driven effects have been seen for child externalizing behavior and adverse parenting styles [39-42], but less is known about these effects for offspring internalizing behaviors. Likewise, an increasing body of research confirms the presence of bidirectional effects (i.e. both parent-driven and child-driven effects) between externalizing behaviors and parenting (e.g., parental negativity and parental conflict) [43, 44]. These findings suggest that parental characteristics can both be caused by, and the cause of, children's internalizing behavior problems although few studies have been able to investigate this effect [15, 45, 46]. Despite a strong association between internalizing problems and different parental characteristics in adolescents, most studies investigating this association have been conducted in childhood [47]. Thus, it is important to note that the mechanisms may differ during development and between different parental characteristics, which make it important to study specific parental characteristics individually and at several time points during the development.

The majority of research exploring the mechanisms underlying the association between parental characteristics and their children's internalizing problems has used cross-sectional study designs (i.e. investigations at one point in time). However, longitudinal studies and studies over generations are needed to clarify the direction of this association. Thus, more information regarding potential bidirectional effects between parental characteristics and internalizing problems is important for understanding the origin and consequences of these behaviors for families [48].

2.3.1 Parenting and internalizing behavior

There is a well-known association between parental practices and internalizing problems in offspring; although most studies have been conducted using cross-sectional designs [49-52]. There are, however, a few studies that have investigated the direction of the association between offspring internalizing behaviors and parenting longitudinally [35, 53]. Findings from a study by Albrecht et al. suggested that adolescents internalizing behavior increased the levels of their parents' psychological control, indicating a child-driven effect. Only a few studies have revealed a bidirectional relationship between internalizing behavior and parenting. For example, in a large sample ranging between age 7 to 12, a bidirectional relationship was seen for girls' depression and low parental warmth (i.e. lack of parental warmth predicted an increase of child depressive problems at the same time as child depressive behavior predicted decreased levels of parental warmth) [15].

One parental measure that has been strongly associated with higher levels of internalizing behavior problems, both in children [50, 52] and in adolescence [34], is parental emotional overinvolvement (EOI). For example, a cross-sectional study suggested that parental EOI was not predictive of any adolescent internalizing behavior [54]. On the contrary, results from the association between offspring internalizing problems and maternal EOI using a design over generations suggested regulations of child-driven effects [34]. These contrasting results indicate that more research is needed to conclude the direction of this association in adolescence.

2.3.2 Psychiatric disorders and internalizing behavior

In addition to parenting, parents' psychiatric disorders have also been associated with an increased risk of internalizing behavior in offspring [55-57]. It is well known that parental depression is particularly related to higher levels of internalizing behavior among offspring [56]. A recent study showed that approximately 17 % of all mothers with young children had increased levels of depressive symptoms [58], which indicates that this is an important public health problem. It is further accepted that offspring of parents with schizophrenia and other psychotic disorders are similarly at an increased risk of internalizing problems in childhood [55]. Likewise, offspring of parents with different forms of psychiatric disorders (i.e. depression, panic disorder, and antisocial behavior) have been linked to an increased risk of suicidal behavior [59-61].

It is also important to acknowledge that children can evoke a certain behavior from their parents, which might result in that they provide an environment that maintains the children's symptoms. The death of a child has been found to increase the risk of psychiatric morbidity, especially affective disorders, among exposed parents [62-64], but whether offspring suicide has an impact on parents' mental health has not been thoroughly investigated [65].

2.4 QUANTITATIVE GENETICS

Quantitative genetics has been used to increase our understanding of how psychiatric characteristics are transmitted from parents to offspring. This field specifically tries to find out the relative influence of genes and environment for individual differences in behavior. Quantitative genetic research is limited to naturally occurring situations, such as adoptions, twinning and different levels of family relatedness. The advantage of these designs is the possibility to control for genetic and environmental factors that are shared between individuals. The designs of comparing differentially exposed relatives have become a valuable tool in trying to disentangle causal effects from familial confounding.

2.4.1 Family-based designs

Different levels of genetic similarities among family members make it possible to study the importance of genetic and environmental factors to specific traits [66-

69]. By comparing relatives we hope to control for genetic and early environmental risk factors shared between individuals.

The twin design is one of the most powerful techniques used in behavioral genetics to estimate the contribution of genes and environment to a specific trait [70]. It compares the phenotypic resemblance between monozygotic (MZ) and dizygotic (DZ) twins. This design makes use of the knowledge that MZ twins are genetically identical while DZ twins share approximately 50% of their segregating genes. A higher correlation among MZ twins than DZ twins suggest the influence of genetic effects.

Comparing the risk of outcome between differentially exposed siblings is another design which tries to account for genetic and early environmental risk factors shared by siblings [71]. Full-siblings are said to share 50% of their segregating genes (since they inherit half of their genes from each parent). Similarly, comparison of differentially exposed half-siblings account for genetic factor inherited from one parent (half-siblings share approximately 25% of their segregating genes) and all environmental factors that make them similar. The importance of shared environmental influences could specifically be investigated by comparing the association among maternal and paternal half-siblings. In Sweden, maternal half-siblings are expected to share more early environments than paternal half-siblings because offspring are predominantly living with their mothers when parents separate [72]. In addition, comparisons of cousins and half-cousins (offspring of half-siblings) are also useful designs to account for some part of familial factors shared within such pairs (cousins and half-cousins share 12.5% and 6.25% of their segregating genes respectively).

In general, if an association is confounded by unmeasured familial factors (e.g. genetic transmission of risk of psychiatric disorders) we would expect the association to be attenuated within exposure-discordant relatives. It is important to note that all natural experiments have their strengths and assumptions, which should be taken into account when interpreting results from family-based designs. Therefore, it is often useful to combine different genetically informative designs to more thoroughly examine causal inferences [66].

2.4.2 Gene environment interplay

A better knowledge of how genes and environment interplay is necessary to fully understand the development of different mental health problems [68]. The process in which genetic influences can control the exposure to the environment is called the gene-environment correlation (rGE) [73]. There are two types of rGE associated with the family environment: evocative and passive. Evocative rGE implies that the child's genotype can evoke an effect of parental behavior [74]. For example, evocative rGE implies that children characterized by increased levels of ADHD (a trait with high heritability) are more likely to receive a more harsh discipline from their parents compared to children without the disorder. However, children's inherited genotypes can also be correlated with the family environment provided by their parents (i.e. passive rGE). For example, parents

with a genetic liability for aggression might transmit some part of the susceptibility genes to their offspring and simultaneously provide a violent home environment due to their own genetically influenced characteristics (Moffit 2005). Knowledge of how genes and environment interplay is important because different parental characteristics might not directly influence offspring behavior but be a spurious association confounded by genetic transmission.

Quantitative genetic studies, such as the classical twins design, have been an invaluable toolbox to study gene-environment correlations. These designs have demonstrated the heritability of various environmental risk factors and been able to disentangle genetic from environmental parts of familial transmission, which is necessary to rule out whether genetic factors account for the association between offspring internalizing behavior and parental characteristics.

2.4.3 Internalizing behavior

Internalizing behavior problems in childhood are highly heritable with genetic factors accounting for 38-74% of the variance in anxiety, depression and withdrawn behaviors [75, 76]. However, the effects of genes and environment have been found to be age specific with decreasing heritability estimates and increased influence of shared environmental influences during adolescence [77]. Thus, the development of internalizing behaviors seems to be influenced by both family environment (such as parenting) as well as genetic factors. Importantly, internalizing behaviors are also stable over time, which indicates that individuals with internalizing problems in childhood are at risk of adversities later in life [5].

Findings from family and adoption studies have similarly shown that genetic factors are important for suicidal behavior [27, 78, 79], even after adjusting for co-occurring psychiatric disorders [80]. Previous twin studies confirm the importance of genetic factors by suggesting heritability estimates for attempted suicide between 30% and 55% [81]. Despite the contribution of previous studies, the mechanisms through which risk factors are transmitted are still poorly understood [59].

2.4.4 Parenting and internalizing behavior

The importance of genetic and environmental influences underlying the association between internalizing behavior and parenting has not been extensively studied. A recent study has provided evidence of rGE on adolescent depressive outcomes [11]. Specifically, genetic effects influenced maternal punitive discipline which further contributed to adolescent depression, implying rGE. These findings indicate that rGE occur in the development of adolescent depressive symptoms and parental punitive discipline.

There are a few studies that have investigated bidirectional associations by using multivariate genetic designs [34, 37, 38]. For example, one previous study indicated that internalizing behavior during adolescence were influenced by former maternal EOI, but also that it was genetically influenced child-characteristics which evoked such behaviors from their mothers, which is

consistent with evocative rGE [34]. Genetic factors have also been found as the primary explanation in the association between depressive symptoms and parental conflict-negativity [38]. In contrast, a previous study showed that non-shared environmental factors had a significant influence in the association between adolescent internalizing behavior and punitive parenting, although genetic factors also are likely to contribute in these processes [37].

2.4.5 Psychiatric disorders and internalizing behavior

It has been difficult to know if the association between parental psychiatric disorders and offspring internalizing behaviors is driven by genetic, environmental factors, or both. There are several different ways that parents with psychiatric disorders could influence offspring internalizing behavior. Children may be influenced indirectly by a familial transmission of psychiatric disorders. Many psychiatric symptoms, such as schizophrenia, suicidal behavior and depression, are partly heritable and have been found to cluster within families [78, 80, 82-84]. Consequently, if psychiatric disorders among parents and offspring internalizing behaviors are influenced by the same genetic effects, a shared genetic liability would create an increased risk of internalizing behavior among offspring of psychiatric morbid parents.

However, the association between parental psychiatric disorders and offspring internalizing behaviors might not be fully explained by common genetic factors. Parents' psychiatric disorders could also affect their children by impaired parenting practices [85-87]. Specifically, parents with schizophrenia could display various behavioral problems such as hostility, agitation and affective symptoms, which most likely would influence their parenting style. In addition, individuals diagnosed with schizophrenia might also provide risky environments, such as completed suicide in non-offspring relatives [88] and domestic violence [89], which might increase the risk of suicidal behavior in offspring [80].

Studying the direction and etiology of parental characteristics and their children's internalizing problems is important to increase our knowledge regarding developmental mechanisms and may inform preventive interventions to ease later negative outcomes.

3 AIMS

The overall objective of this thesis was to investigate the direction and etiology of associations between parental characteristics and offspring internalizing behaviors through the use of different genetically informative designs.

The specific aims were:

- To examine the direction and the etiology of the association between different parenting styles and internalizing behavior problems from adolescence to early adulthood.
- To investigate the impact of offspring death and suicide during adolescence and young adulthood on parental psychiatric morbidity and if such association represents a causal relationship.
- To evaluate the suicide risk among offspring of parents hospitalized for schizophrenia and elucidate the mechanisms behind this association.
- To explore whether ADHD and suicidal behavior share genetic and environmental risk factors.

4 MATERIALS

4.1 SETTINGS

The studies in this thesis are based on data drawn from various Swedish nationwide registers. Paper I is based on data from the Twins study of Child and Adolescent Development (TCHAD), originally derived from the Swedish Twin Register, while Paper II, III and IV used data from a linkage of several different Swedish population based registers. Below is a description of the twin sample and the registers used in this thesis.

4.2 THE TCHAD STUDY

4.2.1 Participants

TCHAD is an ongoing longitudinal study investigating how genes and environment influence the behavior and health development from childhood to early adulthood [90]. The study started in 1994 and includes all 1480 twin pairs born in Sweden between May 1985 and December 1986. The twins and/or their parents have been contacted in four different waves: at age 8-9 (parent only), 13-14, 16-17 and 19-20 (both twins and parents). The response rate was 75% (n=1109) for parent reports in wave 1, 73% (n=1063) for parent reports and 78% (n=2263) for self-reports in wave 2, and 74% (n=1067) for parent reports and 82% (n=2369) for self-reports in wave 3. In wave 4, both parents were approached individually, giving 1158 responses from at least one of the parents (mothers only: n=363, fathers only: n=97, both parent reports: n=698), while self-reports had a response rate of 59% (n= 1705). In Paper I, we used information from wave 3 and wave 4, when the twins were in adolescence and young adulthood. Thus, I will only describe these waves and the measures used in Paper I in more detail.

4.2.2 The representativeness of the sample

Previous reports have shown that responders and subjects lost to follow up at wave 3 did not differ significantly with regards to education level, unemployment level, buying power, and neighborhood crime rate [90]. However, some significant differences were seen for ethnic diversity, indicating a slight underrepresentation of individuals living in neighborhoods characterized by ethnic heterogeneity [91].

In wave 4, previous findings have detected somewhat higher estimates of psychopathic personality and antisocial behavior among non-responders, although the effect sizes were small for these differences [92]. Our own analyses also revealed that subjects lost to follow-up at wave 4 showed slightly higher levels of parent-reported EOI and criticism as well as significantly lower levels of internalizing behavior compared to responders in wave 3.

4.2.3 Zygosity determination

Zygosity was determined by either DNA-tests or by using a zygosity algorithm. DNA confirmation of the zygosity was possible in the last wave when all twins were asked to give saliva, collected with oragene® via mail, whereof 1312 twins provided DNA. The zygosity assignment was otherwise based on a questionnaire of four items, covering the twins' physical similarities, answered at age 8-9 (by parents only) and at age 13-14 and 16-17 (by both parent and twins). Zygosity classification was made for each response individually through algorithms derived from discriminant analyses on 385 twin pairs with known zygosity, which have been confirmed by 47 polymorphic DNA-markers [93]. A final zygosity assignment was set if the pairs had at least 95% probability of being correctly classified as monozygotic (MZ) or dizygotic (DZ) twins. In cases of any contradictions between the five different assignments, the zygosity was set to unknown.

4.2.4 Measures

4.2.4.1 Parental criticism and emotional overinvolvement

Parental emotional overinvolvement (EOI) and parental criticism was assessed using two subscales of the Expressed Emotion (EE) measure [94]. The EE measure assesses different aspects of interpersonal relationships, such as criticism, warmth, positive comments, hostility and emotional overinvolvement [95]. The best method to estimate EE has been the Camberwell Family Interview (CFI), which is a semi-structural interview that covers different aspect of feelings and emotions found in daily family life. However, because the CFI is both time consuming and expensive to complete, different questionnaires to measure EE has been developed. For example, the Family Questionnaire, which is a brief self-report scale, has been shown to assess EE with a 74% agreement to the CFI [96]. In TCHAD, an EE questionnaire, very similar to the Family Questionnaire, which has shown both acceptable reliability and validity, was used [94].

4.2.4.2 Internalizing problems

Internalizing behavior was assessed using reports from both parents and twins. At wave 3, when the twins were 16-17 years old, the parents answered the Child Behavior Checklist, and the twins the Youth Self Report. In the last wave, when the twins were 19-20 years old, the parents answered instead the Adult Behavior Checklist, and the twins the Adult Self Report [7, 8].

4.3 SWEDISH REGISTERS AND LINKAGE

4.3.1 The personal identification number

A unique identification number was introduced in 1947 and is assigned to each Swedish resident directly at birth or at immigration [97]. The number consists of ten digits representing the birth year, month and day, as well as a 4 digit control number. The personal identification number made it possible to link all individuals in Sweden with information from the nationwide registers. However,

before data is received by researchers the true personal identification number is replaced with a unique index number to ensure that all individuals remain anonymous.

4.3.2 The Multi Generation Register

The Swedish Multi-generation Register (MGR) is a unique resource in the world consisting of data on more than nine million individuals and updated yearly [98]. The register links all children (index persons) born in Sweden since 1932 and alive in 1961 to their biological parents and adoptive parents. This holds also for those who emigrated and became Swedish citizens before 18 years of age. From 1961 and onwards the register has excellent coverage with information on 100% of the biological mothers and 98% for the fathers born in Sweden. The coverage is less complete for index persons born outside Sweden, because information on parents are missing if they immigrate older than 18 years of age, and in cohorts older than 1947 (when the national identification number was introduced).

The link between children and parents made it possible to construct large pedigrees of different family relationships. Nearly all index persons have information on at least one parent but more than 47% of the index persons have information on two generations or more. The MGR now holds up to five generations even though the register is far from complete for information of more than three generations.

4.3.3 The National Patient Register

The National Board of Health and Welfare started to collect data to the National Patient Register (NPR) in 1964, initially only including information regarding inpatients care from a few counties in Sweden. From 1987 and onward, the NPR includes records of psychiatric inpatient care with complete national coverage. The register also includes records of outpatient care, from both private and public caregivers, since 2001. All discharge diagnoses, the main discharge diagnosis, and up to eight secondary diagnoses, are recorded according to the International Classification of Diseases (ICD); 8th (1973-1986), 9th (1987-1996), and 10th (1997-2009) revision. The quality of the data in the NPR has been showed to be excellent, with an estimated drop-out rate less than one percent. Previous validation of the inpatient register has also reported high validity for most diagnoses in the register [99].

4.3.4 The Cause of Death Register

The Cause of Death Register includes all deceased persons registered in Sweden at the time of death regardless of whether the death occurred abroad or in Sweden. The register covers nearly all deceased persons since 1952, but is considered complete from 1961 and onward. From the Cause of Death Register we could obtain information the date of death, the cause of death as well as multiple contributory causes of death coded according to the international classification of disease.

4.3.5 The Swedish Prescribed Drug Register

The Swedish Prescribed Drug Register was established in 2005 and provides complete national information on all dispensed prescribed pharmaceuticals. The register comprises drug classifications according to the Anatomical Therapeutic Chemical (ATC), the date of prescription, quantity and dosage, and possible generic substitution. In addition to information on the dispensed drugs, the register includes patient information on age, sex, area of residence and the unique personal identifier to the whole Swedish population. The register is practically complete (with missing data on patient identification number for <0.3% of the population) [100].

4.3.6 The Total Population Register

The Total Population Register was established in 1968 and includes information on name, place of residence, sex, age, civil status, place of birth, citizenship, migration status (from 1969 and onward), and relations for the entire Swedish population.

4.3.7 Database for health insurance and labor market

LISA is a longitudinal integration database for health insurance and labor market studies holding annual registers since 1990. The database contains information from the labor market, educational and social sectors for all individuals registered in Sweden 16 years of age and older.

5 DESIGN AND SUBJECTS

An overview of the designs, participants, and measures for each paper included in this thesis is presented in Table 1.

Table 1. Overview of Paper I-IV

	Paper I	Paper II	Paper III	Paper IV
Study design	Longitudinal design	Cohort design	Nested case-control design	Matched cohort design
Genetically sensitive design	Twin models, cross-lagged and cholesky	Step family design, sibling comparison design	Cousin comparison design	Family design
Study population	Swedish twin pairs born between 1985-1986 contacted at age 16-17 and at age 19-20	All parents in Sweden whose offspring were recorded as alive at the year of their first biological child's 12th birth-year, during follow-up 1960-2008	All individuals with suicidal behavior in Sweden between 12 and 30 years of age born before 1996.	Patients diagnosed with ADHD in Sweden 1987-2009 and their relatives
N	496 MZ twins 356 DZ twins 361 OS twins	3 114 564 parents (277 570 parents within step families, 45 427 full-siblings, 4653 half-siblings)	123 329 case-pairs 594 839 control-pairs (4285 full-sibling 662 half-siblings)	51 707 probands
Outcome	Parental EOI and criticism Offspring internalizing behavior	Parental psychiatric hospitalization	Offspring attempted suicide, completed suicide, and suicidal behavior	Suicidal behavior (attempted or completed)
Exposure	Parental EOI and criticism Offspring internalizing behavior	Offspring completed suicide, and any cause of death	Parental schizophrenia	ADHD
Covariates	Offspring gender	Parental age, parental gender, SES, age at exposure, and nr of children at start of follow-up	Offspring mental illness, parental suicidal behavior, SES	substance use disorder, anxiety bipolar disorder, depression, conduct disorder antisocial personality disorder and schizophrenia

5.1 EPIDEMIOLOGICAL STUDY DESIGNS

One important objective of epidemiological research is to identify the etiology (or cause of disease) for the purpose of better understanding the mechanisms. The most optimal epidemiological design to study causal effects is the randomized control trial, where study subjects are randomized into different exposure groups (for example medical treatment and placebo). This setting provides similar frequency of confounding factors in the two groups, which limits potential bias. The outcome of disease can then be studied among the two different exposure groups to make a statement about causality. However, this design is not always practicable or ethical, especially in psychiatric epidemiology. In this thesis, we have instead used different observational epidemiological designs in combination with genetically informative designs to study causality; these designs will be described briefly below.

5.1.1 The case-control study

In case-control studies we select the source population by the outcome of interest [101]. This design is called a nested case-control study if it is conducted within a clearly defined population [102]. Individuals with the outcome (often a disease) are defined as cases and matched to controls without the disorder. It is important that the control group is selected independently of the exposure of interest in order to avoid bias. For the purpose of Paper II, we used density matching which means that the controls are selected from the population at risk of becoming a case [103]. Thus, the controls could become cases later on. This way of matching ensures equal time at register follow-up within the matched groups.

The measurement of association is usually odds ratio defined as the odds of exposure among cases divided by the odds of exposure among controls. This design is usually retrospective in nature because investigators look back in time to ascertain the exposure status after selection of both cases and controls. However, register-based data could be seen as prospective if the exposure status was registered before the outcome of interest.

In a case-control study it is also possible to additionally match each case to the controls on a variable, usually a strong confounder, to increase efficiency and eliminate this specific confounder between exposure and outcome. However, in order to remove the confounding factor we have to account for the matching in the analyses [104]. The case-control design is preferable when the outcome is rare.

5.1.2 The cohort study

A cohort is defined as individuals followed over a period of time [105]. Unlike case-control studies, the study population in a cohort study is defined by the exposure status. The subjects are drawn from the population at risk of developing the outcome and followed over time to estimate incidence rates of the outcomes of interest. The incidence rate is defined as the number of cases with the disease divided by person-time at risk. Dividing the incidence rate among the exposed

group with the unexposed group determines the incidence rate ratio. A prospective cohort study (i.e. healthy participants are followed over time until outcome or censoring) could be used to provide the direction of effects. The cohort design is much more efficient than the case-control design when the exposure frequency is low, but is generally more expensive and time-consuming than case-control studies.

Matching can also be performed in cohort studies to avoid confounding and increase efficiency. When matching exposed individuals to the unexposed population with regards to a possible confounder, this variable would be the same for the two different exposure groups; hence it cannot confound the association. In practice, this means that the matching does not need to be accounted for in the analyses, as in a case-control study, to prevent bias. The matched cohort design could sometimes share methodological properties with the case-control design, which allows the authors to describe their study in either way.

5.1.3 Longitudinal studies

Many previous epidemiological studies have used cross-sectional designs, which can be described a snapshot of a particular point in time. Although this design can be used to describe an association between exposure and outcome we cannot draw conclusions of causality from such design. Another more useful approach to investigate causes of effects is the longitudinal design, where repeated measurements are taken at multiple time points.

5.2 GENETICALLY SENSITIVE DESIGNS

Research on the relative influence of genes and environment on behavioral differences among humans are limited to naturally occurring situations, such as adoptions, twinning and different levels of family relatedness. The advantage of these designs is the possible to control for genetic and environmental factors shared between individuals which might confound an association. In this thesis we make use of natural experiments such as twins (Paper I), siblings, half-siblings (Paper II, IV), offspring, parents (Paper IV), cousins, half-cousins (Paper III, IV), and stepfathers (Paper II), which will be described in more detail below.

5.2.1 Stepfamily design

In Sweden, offspring usually continue to live with their mothers when parents separate [72]. Therefore, stepfathers would share the same family environment as their stepchildren, but they would not be biologically related. This quasi-experimental design is one way to try to investigate if an observed association represent a causal relationship.

In paper II, we used stepfathers exposed to the death of their biologically unrelated stepchild to disentangle the mechanisms behind the association between offspring death and psychiatric disorders among bereaved parents. If we observed an increased risk of psychiatric disorders among stepfather this

would support a causal environmental relationship. In contrast, the lack of a significant association among stepfathers may be interpreted as underlying genetic influences, although this could also indicate that the death of a biologically unrelated child might be less stressful compared to the loss of a biological child.

5.2.2 Sibling comparison

The sibling design estimates the risk of outcome within exposure discordant pairs in an attempt to control for genetic and environmental confounding shared by the siblings.

In paper II, we used the sibling comparison design to understand the mechanisms underlying the association between offspring death and parental psychiatric disorders, by analyzing all sibling pairs discordant for exposure of offspring death. In this setting, a substantial attenuation of the relative risk among differentially exposed full-siblings (or half-siblings) may indicate familial confounding, but it could also be explained by a causal effect that cut across the extended family. That is, siblings to parents who lost an offspring could to some extent also be influenced by the death of their niece/nephew.

5.2.3 Cousin comparison

The cousin comparison design is another tool to disentangle familial confounding from causal environmental mechanisms, by comparing the risk of outcome found within exposure discordant full-cousin and half-cousin pairs with the risk of outcome in the population.

In paper III, we explored the importance of familial risk factors by comparing the risk of suicidal behavior within full-cousins, and half-cousins (whose parents are half-siblings) differentially exposed for a parent with schizophrenia. There could be two different patterns of results in the cousin comparison analyses. First, if the suicide risk within differentially exposed full- and half-cousins remained at the same level as that within the unrelated general population, this would indicate an environmental association. Second, if the risk of suicide within differentially exposed full-cousins was diminished compared to the risk in half-cousins, the results would indicate that the association is confounded by genetic mechanisms; because full-cousins share 12.5% of their segregating genes while half-cousins only share 6.25%. Correspondingly, if genetic effects are important, we expect the suicide risk among half-cousins differentially exposed for parental schizophrenia to be somewhat attenuated compared to the risk obtained with population controls.

5.2.4 Family design

A family design makes it possible to study the risk pattern for an association of interest across different levels of relatedness. Thus, comparing the risk estimates among first-degree relatives (e.g. offspring and parents), second-degree relatives

(e.g. half-siblings), and third degree relatives (e.g. cousins) can provide evidence for genetic and environmental influences on an observed association.

This design was used in paper IV to study the underlying mechanisms between the comorbidity of ADHD and suicidal behavior. An increased risk among first-degree relatives compared to the risk among more genetically distant relatives indicates shared familial factors for the observed association. Additionally, comparing the risk estimates among maternal half-siblings and paternal half-siblings would indicate the importance for shared environmental influences. Specifically, because offspring usually continue to live with their mothers when parents separate maternal half-siblings are assumed to share more family environment than paternal half-siblings. Thus, if shared environmental influences are important we would expect higher risk estimates among maternal half-siblings.

5.3 PAPER I-IV

Paper I

We performed a longitudinal study with a genetically sensitive design to investigate the direction and etiology of the association between two different parenting styles and internalizing behavior.

In this paper, we included data from the TCHAD-study where all Swedish twins, born between 1985 and 1986, were contacted at 16-17 and 18-19 years of age. The final sample was restricted to twins with known zygosity and information from at least one informant (self-, mother-, or father-reports). In all, we included 2426 participants consisting of 496 MZ twins, 356 DZ twins and 361 opposite sex twins.

Classifications

Participants in TCHAD were asked to answer questions regarding parenting and internalizing behavior. The measures were created by items in common for both time points and all scales. As a result one item was deleted from the measure of Parental EOI and 4 items from the internalizing behavior scale. The final measure of parental EOI consisted of 7 items (e.g., "I can't sleep because of him/her"), and refers to an excessively self-sacrificing and over-intrusive behavior towards a family member. Parental criticism consisted of 10 items (e.g., "he/she irritates me"), and refers to critical comments about the behavior of a family member. All items were rated on a five-point scale (where 1= never and 5= always). The majority of the parent reported information (83%) at wave 3 was provided by the mother, while at age 19-20, each parent responded individually. We combined informants to minimize possible reporter bias and to obtain more comprehensive measures of behavior. Therefore, the final composite measures of parental criticism and parental EOI at age 19-20 were obtained by averaging the standardized scores from mother and father reports.

Internalizing behavior problems was assessed using reports from both parents and twins via 25 items from the three empirical subscales anxious/depressed (e.g., “self-conscious or easily embarrassed”), withdrawn/depressed (e.g., “would rather be alone than with others”), and somatic complaints (e.g., “overtired without good reason”). At wave 3, we averaged the scores from self-reports and parent-reports, allowing for one informant to be missing. Similarly, the internalizing measure at wave 4 was created by first averaging mother- and father-reports of internalizing behavior, and then averaging with the self-report. The reliability of the reduced scales of parental EOI, parental criticism, and internalizing behavior, measured with Cronbach’s alpha, was acceptable for all informers at both waves ($\alpha > 0.7$).

Paper II

We applied a prospective cohort study to investigate the impact of offspring any death and completed suicide during adolescence and young adulthood on subsequent parental psychiatric morbidity.

A record linkage of Swedish nationwide registers resulted in a cohort of 3 114 564 parent-offspring pairs born between 1932 and 1996. The cohort was restricted to parents whose offspring were recorded as alive on their first biological child’s 12th birthday. These healthy parents were followed until the end of 2008, which resulted in 151 076 first-time hospitalizations for psychiatric disorder.

To address possible confounding of genetic and environmental factors we applied two family based analyses. First, we identified stepfamilies from mothers having children with two different fathers. Similar to the total parental sample, the cohort of stepfamilies only included stepfathers whose stepchildren were alive at the oldest stepchild’s 12th birthday, and whose (if any) biological offspring had survived at least until 25 years of age. This resulted in cohort of 277 570 biological mothers and stepfathers. Second, we also used the interlinked registers to identified differentially exposed full-siblings (10 527 for suicide and 45 427 for any death) and half-siblings (1211 for suicide and 4653 for any death). Again, all sibling pairs had to have their first offspring alive on his/her 12th birthday.

Classifications

The NPR provided information on in-patient discharge diagnoses of psychiatric disorders, such as affective disorders (bipolar disorder, depression and affective personality disorders) and substance use disorders (alcohol and drug abuse or dependence). The Cause of Death Register made us identify all deceased persons between 1969 and 2008, including completed suicides. To investigate the effect of offspring any death and completed suicide during adolescence and young adulthood we restricted these events to occur between 12 and 25 years of age. Suicidal behavior is exceedingly rare before 12 years of age and this age was set as a lower limit. The upper cut-off at 25 years of age was chosen to increase the probability that the offspring quite recently had lived together with their parents. Information on parental age, gender, education, and number of children

at start of follow-up were retrieved from register linkage. To simplify the models we used the parental mean age in each family. The parental mean age at suicide was defined as years deviating from the mean. Educational level was categorized into elementary education (<10 years), secondary education (10-12 years) and higher education (>12 years). The number of children at start of follow-up was likewise divided into three groups; one child, two children and three or more children.

Paper III

We conducted a nested case-control study within Swedish population-based registers to investigate the risk of suicidal behavior among offspring whose parents were hospitalized for schizophrenia. This design was considered appropriate when studying rare events such as suicidal behavior.

Linking index persons in the MGR and the Total population register made it possible to identify almost 14 million child-parent relationships, with children born between 1932 and 1996. We used the Cause of death register and the NPR to identify 68 318 unique cases with suicidal behavior (attempted or completed suicide) between 12 and 30 years of age. Linking each suicide case with their known biological mother and father resulted in 123 329 offspring-parent pairs. To each suicide case-parent pair we matched five pairs of offspring-parent controls on birth-year and gender which resulted in 594 839 offspring-parent controls.

To study possible familial confounding, we also applied a cousin comparison design. Again, we used the interlinked population-based registers to identify all parental sibling pairs (both full-siblings and half-siblings) discordant for schizophrenia. To increase homogeneity with the original nested case-control study we excluded offspring born after the end of 1996, and also twin parents because monozygotic twins are genetically identical. In all, 4285 pairs of full-siblings and 662 pairs of half-siblings discordant for schizophrenia and their children were used in the analyses.

Classifications

From in-patient care in the NPR we identified suicide attempts and offspring mental illnesses (i.e. schizophrenia, other non-organic psychotic disorder, bipolar disorder, affective, anxiety, phobic, obsessive, dissociative, somatoform, substance use, and personality disorder). Offspring were regarded having a suicide attempt if it was recorded as main or secondary diagnoses in the register. However, any in-patient diagnose was enough for offspring to be defined as having other mental illnesses. Completed suicides (both definite and uncertain) were found in the Cause of death register. Information on SES was retrieved from LISA and assessed by highest attained education level in the parent. Again, the educational level was categorized into three groups.

Paper IV

A matched cohort design was applied to estimate the relative risk of suicidal behavior among individuals diagnosed with ADHD (probands). Linkage of

Swedish nationwide registers using the unique personal identification number as key, made it possible to identify 51 707 patients with ADHD in Sweden between 1987 and 2009 and 258 535 controls matched on gender and birth-year.

Similar to the previous papers we had to use a family-based design in order to investigate the importance of familial factors. We estimated the relative risk of suicidal behavior across different levels of genetic relatedness. For each type of relationship (parents, full-siblings, maternal- and paternal half-siblings, and cousins) we matched each ADHD proband and his or her exposed relative to five healthy controls and their corresponding relatives. Individuals were matched on gender and birth-year as an attempt to eliminate confounding, reduce misclassification of exposure and to ensure equal time at register follow-up. Several pairs could be descended from one proband (e.g. if a proband has several siblings), and each individual in the population could appear in multiple relative groups (e.g. parent, siblings). Controls were chosen among individuals who were alive, living in Sweden and not diagnosed with ADHD at the year of the proband's first ADHD diagnose.

Classifications

Patients with a discharge diagnosis of ADHD were identified from in-patient or out-patient care in the NPR. We also identified individuals treated with medication for ADHD from the Prescribed Drug Register, and defined them as ADHD patients. Individuals with an ADHD diagnose between 3 and 40 years of age were selected as probands.

Suicidal behavior was defined as any record of suicide attempt or completed suicide (including both definite and uncertain diagnoses) from the NPR and the cause of death register, respectively. To reduce possible misclassifications, suicidal behavior was only allowed in individuals 12 years or older. The NPR also provided information about substance use disorder, depression, anxiety, conduct disorder, bipolar disorder, schizophrenia and antisocial personality disorder. In line with previous register-based research we used a hierarchical approach to define psychiatric covariates [106]. Thus, individuals registered with bipolar disorder, but not schizophrenia, were regarded as diagnosed with bipolar disorder. Similarly, individuals having depression were not allowed with co-occurring diagnoses of schizophrenia or bipolar disorder, and individuals having anxiety were not diagnosed with schizophrenia, bipolar disorder or depression.

6 STATISTICAL ANALYSES

6.1 QUANTITATIVE GENETIC METHODS

6.1.1 The classical twin design

The classical twin design assumes that the variance of an observed phenotype (P) can be partitioned into additive genetic (A), dominance genetic (D), shared environmental (C), and non-shared environmental (E) effects.

$$\sigma_P^2 = \sigma_A^2 + \sigma_D^2 + \sigma_C^2 + \sigma_E^2$$

The additive genetic value refers to the summarized effect of all alleles across loci, while dominance genetic effects are non-additive and expressed as interactions between alleles at the same locus. Shared environmental effects refer to environments that increase similarity within a twin-pair, whereas non-shared effects refer to environments that make twins different from each other, including measurement errors [74].

The proportion of phenotypic variance in a population that is attributable to genetic variation is called the heritability (or narrow-sense heritability) and is a frequently used concept in epidemiology [107]. The heritability attributable to additive genetic effects is sometimes termed narrow sense (σ_A^2/σ_P^2), whereas the proportion of variance attributable to additive and non-additive effects ($(\sigma_A^2 + \sigma_D^2)/\sigma_P^2$) has been referred to as broad-sense heritability [74].

A path diagram is a way to visualize a structural equation model. Figure 3 presents a path diagram of the basic twin model of one phenotype, which illustrates how the phenotypic variance is partitioned into effects of A, C and E.

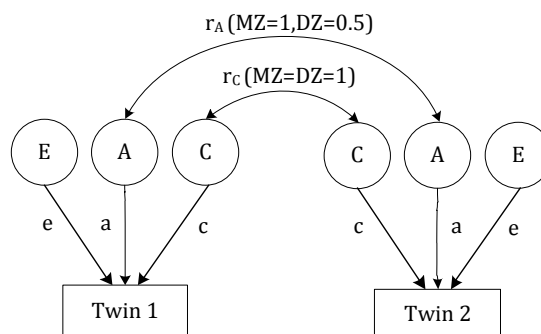


Figure 3. The classic ACE twin model for one phenotype. r_A : genetic correlation; r_C : shared environmental correlation.

Variables in shape of a square are observed variables, while circular shapes describe latent variables (non-measured values). Single headed arrows from latent to observed variables represent causal pathways, while double headed arrows describe the covariance between two variables.

The model assumes that the genetic correlation (r_A) is 1.0 between MZ pairs and 0.5 between DZ pairs. The shared environmental correlation (r_C) is set to 1.0 (MZ and DZ pairs are assumed to experience the shared environment to the same degree), and the correlation between non-shared environments is 0.0 by definition. In addition, the model assumes that genes and environments do not co-vary and there is no interaction between them.

The path estimates (a , c , e) are regression coefficients which in this model estimate the effect of latent variables on the observed phenotype. The path estimates are often standardized (divided by the phenotypic variance) which allows for the comparison of the relative magnitude of different genetic and environmental effects. The relative contribution of effects can be calculated by following path tracing rules [108]. Thus, the phenotypic variance (σ_P^2) and covariance between twins can be expressed as follows:

$$\sigma_P^2 = a^2 + c^2 + e^2$$

$$COV_{MZ} = \begin{bmatrix} a^2 + c^2 + e^2 & a^2 + c^2 \\ a^2 + c^2 & a^2 + c^2 + e^2 \end{bmatrix}$$

$$COV_{DZ} = \begin{bmatrix} a^2 + c^2 + e^2 & \frac{1}{2}a^2 + c^2 \\ \frac{1}{2}a^2 + c^2 & a^2 + c^2 + e^2 \end{bmatrix}$$

Note that the classical twin design cannot estimate the A and D components at the same time because the number of parameter (σ_A^2 , σ_D^2 , σ_C^2 , σ_E^2) would be greater than the number of statistics (σ_P^2 , r_{MD} , r_{DZ}) which prevents the possibility of solving all linear equations [109].

6.1.2 Model fitting

Structural equation modeling (SEM) is used to find parameter estimates within a specified model that explain the variance and covariance of the observed data [110]. SEM has the possibility to use a combination of path analysis, factor analysis and linear regression and is further capable of accessing global fit statistics of complex models including many linear equations [111].

Mx is a commonly used statistical package for twin model fitting [112]. Mx allows models to be fitted to raw data using raw maximum likelihood estimation, which makes it possible to include twin pairs with missing data on one informant. It is also possible to test if two nested models (i.e. the parameters of one model are a subset of the parameters in another model) are statistically significant. The goodness of fit statistics between two nested models are provided through a likelihood ratio χ^2 -test. The goodness of fit of different models, not necessarily nested, could also be compared by the Akaike's information criterion (AIC, χ^2 -2df) or the Bayesian Information Criterion (BIC). To show a good fit the reduced model is supposed to have a non-significant χ^2 -distribution at some user-specified p-value. In addition, a model with lower AIC or BIC was considered to have better fit to the data [113].

6.1.3 Multivariate genetic modeling

The classical twin model can easily be extended by adding more variables. In Paper I, we used the multivariate quantitative genetic models described below.

6.1.3.1 Cross-lagged model

The cross-lagged model is able to analyze the genetic and environmental covariance between multivariate phenotypes over time (Figure 4). All phenotypic associations across time-points (b_{11} , b_{22} , b_{12} and b_{21}) are expressed as partial regression coefficients, and in doing so the model has the advantage of simultaneously estimating the strength of the longitudinal phenotypic associations between two phenotypes when controlling for preexisting association between them.

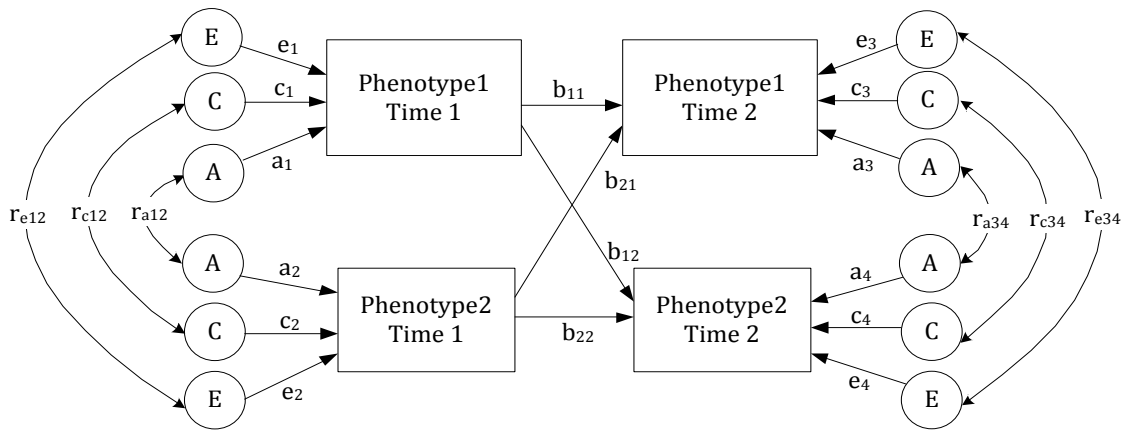


Figure 4. Path diagram of the cross-lagged model. Observed variables are depicted in squares. Circular shapes depict the latent variables A (additive genetic), C (shared environment) and E (non-shared environment). Included in the model are also standardized path estimates and correlations between the latent variables.

In addition, the model can be used to estimate the genetic and environmental contribution of the total variance at follow-up [43]. For example, following path tracing rules the total genetic variance of parenting at time 2 is calculated as follows:

$$\sigma_{Total\ genetic\ P2T2}^2 = (b_{12}^2 * a_1^2) + (b_{22}^2 * a_2^2) + 2 * (b_{12} * a_1 * r_{a12} * a_2 * b_{22}) + a_4^2$$

The variance can then be decomposed into four different effects as shown below:

Child-driven effects: $(b_{12}^2 * a_1^2) / \sigma_{Total\ genetic\ P2T2}^2$

Stability effects: $(b_{22}^2 * a_2^2) / \sigma_{Total\ genetic\ P2T2}^2$

Common effects from time 1: $2 * (b_{12} * a_1 * r_{a12} * a_2 * b_{22}) / \sigma_{Total\ genetic\ P2T2}^2$

Residual effects at time 2: $a_4^2 / \sigma_{Total\ genetic\ P2T2}^2$

6.1.3.2 Sex-limitation model

The sex-limitation models enable tests for qualitative sex differences, quantitative sex differences, and phenotypic variance differences between genders. Qualitative genetic effects imply that different genes operate on the behavior in each sex and are suggested when the genetic correlation between opposite-sex DZ is less than 0.5. Quantitative sex differences refer to dissimilarity in the magnitude of genetic and environmental effects and are indicated when the correlation between twins is different for boys and girls of similar zygosity.

Quantitative sex-differences can be tested by a sex-difference model which allows for different parameter estimates of genetic and environmental effects for each gender. The scalar-model is used to tests for phenotypic variance differences between boys and girls, but forces the proportion of variance accounted for by genetic and environmental factors to be equal across gender. Qualitative sex-differences can be tested by an entirely constrained model, where all parameters for boys and girls are equated (i.e. no differences)

6.1.3.3 Cholesky decomposition

The Cholesky decomposition is used to decompose variation and co-variation into uncorrelated components. Specifically, any positive definite matrix are decomposed into triangular matrices (i.e. have zeros in all components above the diagonal) [112]. Figure 5 shows the path diagram of the Cholesky decomposition for three phenotypes.

In contrast to the cross-lagged model, the Cholesky decomposition is unable to estimate the strength of bi-directional associations, but instead it allows for specific estimations of the genetic and environmental contribution to the cross-lagged effects.

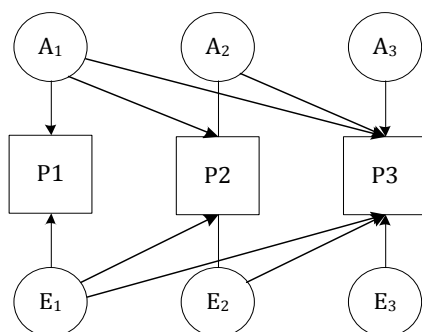


Figure 5. Trivariate Cholesky decomposition. The figure only includes additive genetic (A) and non-shared environmental (E) variance for a more perspicuous representation.

6.2 MEASURES OF EFFECT

In epidemiological studies we use either relative or absolute measures to estimate the magnitude of the association between an outcome and exposure. It is important to understand the difference of these measures because they provide different information to public health policy makers.

The relative risk or risk ratio (RR) measures the strength of an association and is defined as the probability of having the outcome in the exposed group compared to the probability of having the outcome in the unexposed group. The RR is often easier to relate to and is therefore regularly expressed as an incidence rate ratio (IRR), hazard ratio (HR), or odds ratio (OR). However, the odds ratio is only a good approximation of the relative risk when the prevalence of the outcome is rare. The importance of the RR could easily be misinterpreted. For example, RR of completed suicide among exposed individuals could be extremely high but only affect a very small proportion of the population.

Absolute measures are more informative regarding the impact on population level, especially when the outcome of interest is rare. A commonly used absolute measure is the risk difference, where the risk among exposed and unexposed individuals are subtracted instead of divided (as in the ratio). The absolute risk has in this thesis been measured through incidence proportion (number of new cases during a certain period divided by individuals at risk of developing the outcome) and prevalence (proportion of cases in the population at a certain time period).

6.3 GENERALIZED LINEAR MODELS

The class of generalized linear models (GLM) provides a general framework for many statistical models, such as linear regression, logistic regression and Poisson regression. The GML describes the mean outcome as a linear function of a set of predictors, and uses a link function to explain the relationship between them. Thus, the GML handles binary and ordinal outcomes by transforming the outcome variable through the link function (instead of requiring that the outcome variable itself must vary linearly).

6.3.1 Logistic regression

The logistic regression model is applied when outcome variable is binary (e.g. death or disease) [114]. This model makes use of a logit function to describe the probability event as a linear function of one or several predictors.

$$\text{Logit}(\pi) = \log\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k$$

π = the probability of having the outcome

$(\pi/1-\pi)$ = the odds

X_1 - X_k = explanatory variables

β_0 - β_k = regression parameters

The interpretation of a logistic regression coefficient is similar to other regression models; for example, a change of one unit in X_1 gives a β_1 unit change in the log-odds when all other coefficients are held constant. Thus, in order to interpret the effect as odds we have to transform the coefficient from log-scale to a linear scale by exponentiation [115].

Conditional logistic regression can be used to handle matched or stratified data [105]. This model makes comparisons between exposed and non-exposed individuals within each stratum separately. We used this model for sibling comparisons and matched case-controls designs.

Clustered data, e.g. individuals from the same family, are likely to be positively correlated and have to be handled properly in the analysis to avoid bias [116]. One way to model clustered data is through the sandwich estimator which provides robust standard errors of such data [117].

We used the PHREG procedure with the COVSANDWICH and ID statement to conduct conditional logistic regression model on clustered data in SAS.

6.3.2 Poisson log-linear regression

Poisson regression is designed for modeling count data. These models usually employ the natural logarithm as a link function and express the event of interest as a linear function of a set of explanatory variables. Thus, to estimate the rate of the occurrence of events we used the log-linear Poisson regression model as follows:

$$\text{Log}(\mu) = \log(t) + \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k$$

μ = expected events

t = person time

X_1 - X_k = explanatory variables

β_0 - β_k = regression parameters

The $\log(t)$, sometimes called an offset variable, was used to adjust for differences in follow up time. The model assumes that the events are independent, and occurs approximately at random. Under this model, the anti-logarithms of the regression coefficients in the model are equal to the incidence rate, except for the exponent of β_0 which is the predicted rate at baseline. The parameters from Poisson regression modeling are adjusted for the underlying time scale, which makes it important to choose the most relevant time scale for your research question. The time scale is usually split into finer intervals assuming that the rate is constant within these time periods.

Poisson regression models were performed using the GENMOD procedure in SAS, together with the REPEATED statement to obtain robust standard errors.

6.4 COX PROPORTIONAL HAZARD REGRESSION

The Cox proportional hazard regression might be the most widely used method for time to event data, but is similar to Poisson regression models [118]. The Cox regression is based on the assumption of proportional hazards over follow-up time, an assumption which is not necessary to make in Poisson regression. If the proportional hazards assumption is violated for different levels of a variable it is possible to allow separate hazards for different strata, a procedure known as the stratified Cox model.

In the Cox model, the underlying time scale is incorporated into the baseline hazard and cannot be estimated, which makes the model very efficient. However, Poisson regression is preferred when we want to include several different time scales, which can be difficult to handle in Cox regression. The Cox regression and the Poisson regression would produce the same results if each event in a Poisson regression were split into separate time intervals [118]. The measure of effects from a Cox regression is interpreted as hazard ratios.

We performed a stratified Cox proportional hazards model in SAS using the PHREG procedure with the STRATA statement.

6.5 PAPER I-IV

Paper I

We used the statistical package Mx to carry out the twin model fitting. All scales were \log_{10} -transformed to correctly fit as multivariate normal. We used a cross-lagged model to investigate time-dependent genetic and environmental effects and bidirectional associations across time.

First, we tested potential sex-differences by comparing the fit of the full sex-limitation model with three nested models (the sex-difference model, the scalar-model and the constrained model). The model with lowest BIC was chosen as the best fitting model. Next, we also tested the significance of the cross-lagged parameters by omitting these regression parameters one at a time. To find the best fitting model we compare the $-2\ln$ between the full model and the restricted nested model. The parameter estimates from the cross-lagged model was used to decompose the total genetic and environmental variance of the measure at age 19-20 into four different effects; cross-lagged effects, stability effects, the common effects from age 16-17, and residual effects at age 19-20

In addition, a trivariate Cholesky decomposition was also applied to the data in order to investigate the contribution of genetic and environmental influences on the cross-lagged effect.

Paper II

We estimated incidence rate ratios of psychiatric disorders between exposed parents and non-exposed parents by fitting a log-linear Poisson regression model to the data. We followed all parents from the year of their first offspring's

12th birthday until the year of first diagnosis of psychiatric disorder, death, emigration, a follow-up time of more than 25 years or the end of 2008 whichever came first.

Poisson regression models were performed using offspring age as the underlying timescale. We used person-years as an offset variable in the models and handled correlation between the observations within each family through standard generalized estimating equation methods to obtain robust standard errors. All models were adjusted for mean parental age together with time (five year periods) since start of follow-up, parental sex, highest attained education level among the mother and the father, parental mean age at suicide, and number of children at start of follow-up. To evaluate if the risk of parental psychiatric hospitalization could be entirely explained by suicide we performed separate analyses for the exposure of suicide and any cause of death in offspring. The effect of parental gender and different psychiatric diagnoses were also performed using subgroup analyses.

A Poisson regression model, as described above, was also applied to a sample of stepfamilies. The only difference was that stepfathers were followed with regards to their stepchildren. In addition we also fitted a stratified Cox proportional hazard regression model to a sample of siblings differentially exposed to offspring death. We stratified this model on family id (i.e. each strata is allowed to have its own hazard) to take into account an expected positive correlation between siblings from the same family.

Paper III

We estimated odds ratios (with 95% confidence intervals) of suicidal behavior among offspring exposed to parents with schizophrenia by using conditional logistic regression with a robust sandwich estimator. We calculated crude ORs between cases and matched controls, as well as odds ratios adjusted for SES, parental suicidal behavior and offspring mental illness. Additionally, the analyses were stratified by offspring age at suicide (12–18 years, 19–25 years, and 26–30 years), and gender of the parent suffering from schizophrenia.

Similarly, the difference in exposure between cousin pairs was analyzed using conditional logistic regression in combination with a robust sandwich estimator to handle correlated data.

Paper IV

We used conditional logistic regression including a robust sandwich estimator to estimate odds ratios of suicidal behavior within individuals diagnosed with ADHD, and among relatives of ADHD probands. Separate models were fitted for attempted and completed suicide.

We further conducted two sensitivity analyses. First, to explore if the results were robust, we estimated the risk of suicidal behavior among relatives after excluding ADHD probands with suicidal behavior and relatives with an ADHD diagnose

[119]. Second, we also performed the analyses after excluding individuals with substance use disorder, depression, anxiety, conduct disorder, bipolar disorder, schizophrenia, and antisocial personality disorder.

7 RESULTS

7.1 PAPER I

Parent-effects, child-effects or both?

We found that the cross-lagged model with sex-differences was the best fitting model for these associations. First, we explored the association between parental EOI and internalizing behavior. The results revealed child-driven effects underlying the relationship between parental EOI and internalizing behavior in girls. Specifically, we found that the child-driven effect from daughters' internalizing behavior at age 16–17 explained 2.1% (0.146^2) of the total variance in parental EOI at age 19–20, whereas no bidirectional effect was found for boys (Figure 6). The association between parental criticism and internalizing behavior showed no directional effect for either boys or girls. We therefore focus the remaining part of the result section on the significant child-driven effect observed among girls.

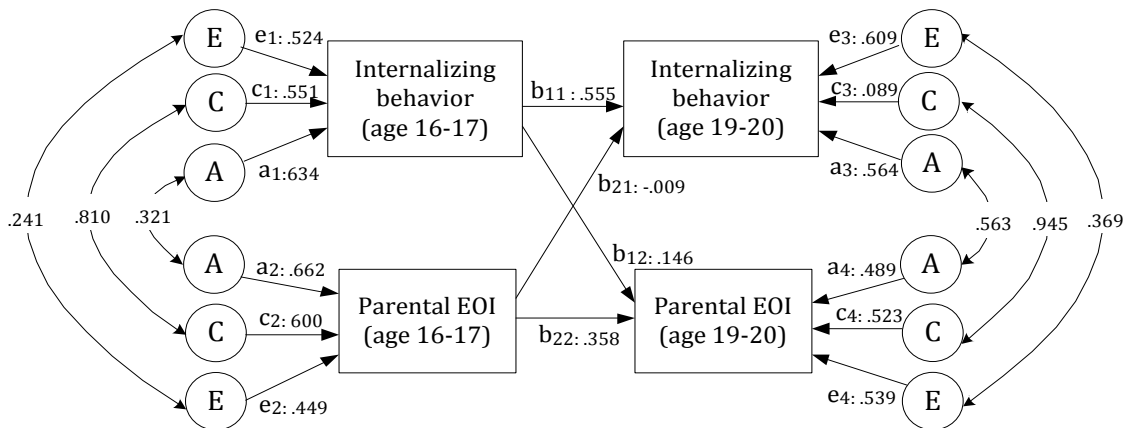


Figure 6. Standardized path estimates for the cross-lagged model for parental EOI and internalizing behavior in girls.

Genetic and environmental contributions

Next, we decomposed the genetic and environmental contribution of the total variance of parental EOI at age 19–20 into four different effects; child-driven effects, stability effects, common effects from age 16–17, and residual effects at age 19–20. The total genetic, shared environmental and non-shared environmental variances for parental EOI are shown in the first row of Table 2 (A=31.8%, C=35.4%, E=32.9%). I here only discuss the child-driven effect in detail. The genetic effect from internalizing behavior at age 16–17 explained 2.7% ($[(0.146^2 * 0.634^2) / 0.318]$) of the total genetic variance in parental EOI at age 19–20. Similarly, the shared environmental effect from internalizing behavior at age 16–17 explained 1.8% of the total shared environmental variance in parental EOI at age 19–20, whereas the non-shared environmental effect for internalizing at age 16–17 explained 1.9% of the total non-shared environmental variance in parental EOI at age 19–20.

Table 2. Percentages of variance accounted for in girls for parental EOI

Proportion of variance due to:	Parental EOI at age 19-20			
	Total Phenotypic variance (%)	Total ACE (%)		
		A	C	E
Stability effects	100	31.8	35.4	32.9
Cross-lagged effects	12.8	17.7	13.0	7.8
Common effects at age 16-17	2.1	2.7	1.8	1.9
Residual effects at age 19-20	4.8	4.4	7.9	1.9
	80.3	75.2	77.3	88.4

Note. A: genetic factors; C: shared environmental factors; E: non-shared environmental factors

Follow-up analyses of the child-driven effect in girls

We further used a trivariate Cholesky design to specifically explore the genetic and environmental impact on the cross-lagged coefficient. The contribution of internalizing behavior on parental EOI, after controlling for the stability in parental EOI is shown within the square in Figure 7. The exclusion of the genetic component resulted in a significant decrease in fit (a_{23} : $\Delta\chi^2=7.15$, $\Delta df=1$, $p=0.01$), while dropping both the shared and the non-shared component resulted in a non-significant deterioration in fit (c_{23} and e_{23} : $\Delta\chi^2=0.40$, $\Delta df=2$, $p=0.82$). Thus, as seen in Figure 7, the partial correlation coefficient (0.10; 0.574×0.172) was entirely explained by genetic factors. In addition, residual effects for parental EOI at age 19-20 accounted for 72% ($0.417^2 + 0.519^2 + 0.527^2$) of the variance, which is in line with the results generated from the cross-lagged model.

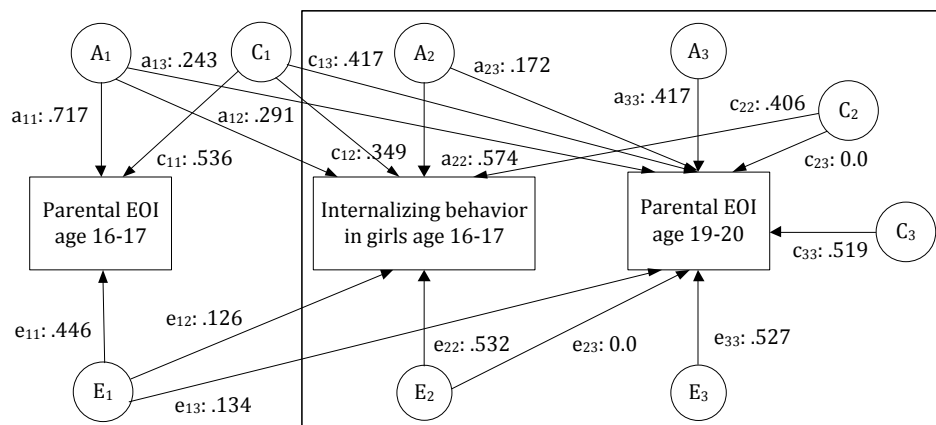


Figure 7. Path diagram of a trivariate Cholesky decomposition. The contribution of child internalizing behavior on parental EOI, after controlling for the stability in parental EOI is shown within the square

7.2 PAPER II

Characteristics of the participants

We followed a cohort of 3 114 564 parents (of whom 51.8% were mothers) for 53 212 181 person-years and found 151 076 first-time hospitalizations for psychiatric disorder. During the follow-up we also registered 3284 suicides and 14 095 any cause deaths among the offspring. In total 1.3% of the cohort were censored due to emigration or death. Parents exposed to offspring death and suicide did not differ much compared to unexposed parents, although unexposed parents had somewhat higher education and fewer children than exposed parents did

Main findings

Parents exposed to offspring suicide had highest risk of subsequent psychiatric hospitalization, even after adjusting for covariates (Table 3; RR=1.90; 1.72-2.09). The risk associated with offspring any death was considerably lower (RR=1.34; 1.27-1.41), and even further attenuated after excluding suicide from any cause of death in offspring (RR=1.18; 1.11-1.26). The same pattern was observed in analyses stratified by type of psychiatric diagnosis (affective and substance use disorder) and parental gender.

Table 3. Relative risk of any psychiatric disorder among Swedish parents, 1960-2008, following exposure to offspring suicide or death

Exposure status	No. of admissions	Person years	Relative risk ^a (95% CI)
Offspring suicide			
Exposed	427	78 112	1.90 (1.72-2.09)
Unexposed	150 649	52 682 064	1.00
Offspring death			
Exposed	1461	377 542	1.34 (1.27-1.41)
Unexposed	149 615	52 382 631	1.00
Offspring death (excluding suicide)			
Exposed	1036	299 888	1.18 (1.11-1.26)
Unexposed	150 041	52 460 252	1.00

^aRisk estimates were adjusted for mean parental age, gender, educational level, age at exposure, and number of children at start of follow-up.

Stepfamilies

Similar to the population based sample, we found that mothers in the subsample of stepfamilies (who experienced the suicide of a biological child) had an increased risk of psychiatric hospitalization (RR=2.49; 1.80-3.44). These results were consistent for exposure of any cause of death. In contrast, we found no risk increase among stepfathers differentially exposed to their biologically unrelated stepchildren's suicide (RR=0.89; 0.53-1.48) or death (RR=0.93; 0.68-1.20). These results indicate that there might not be a direct effect of offspring death on parental psychiatric hospitalization.

To test the robustness of the results from the stepfamily design we applied two sensitivity analyses. First, we repeated the stepfamily analyses using only definite completed suicide as exposure to detect potential effects of the exclusion of an uncertain measure of completed suicide. Second, to increase the chance that stepfathers had become emotionally attached to their stepchildren, we repeated the analyses using stepfathers involved since the stepchild was seven years of age (reflective of longer time to become attached). The results from these sensitivity analyses revealed similar risk estimates as the sub-cohort of all stepfamilies.

Sibling comparisons

The risks for psychiatric hospitalization among full-siblings exposed to offspring suicide (OR=1.35, 95% CI 1.09-1.66) and any death (OR=1.20, 95% CI 1.08-1.34) were noticeably lower than the risk estimates in the total cohort of parents (Table 4). The attenuation of the relative risk in exposure-discordant full-siblings further indicates that the association might be explained by a shared genetic liability for psychiatric disorder rather than a causal environmental mechanism. However, we did not have enough power to draw conclusions from the risk of psychiatric hospitalization among differentially exposed half-siblings.

Table 4. Adjusted relative risks for any psychiatric disorder among full- and half-siblings, 1960-2008, differentially exposed to offspring suicide or death

Sibling type/ Exposure	Nr. of pairs	Relative risk (95% CI)
Parental full-siblings differentially exposed to:		
Offspring suicide	10 527	1.35 (1.09-1.66)
Offspring death	45 427	1.20 (1.08-1.34)
Parental half-siblings differentially exposed to:		
Offspring suicide	1211	1.80 (0.94-3.43)
Offspring death	4653	1.08 (0.78-1.50)

7.3 PAPER III

Characteristics of the participants

In total, we included 68 318 cases with suicidal behavior (12.4% completed suicide and 90.2% suicide attempts) between 12 and 30 years of age, and their matched controls. A history of mental illness was more than ten times higher among suicidal behavior cases compared with controls. Cases had also somewhat lower education level than controls.

Main findings

The main findings of Paper III are presented in Table 5. We found a statistically significant increased risk of suicidal behavior in offspring of parents with schizophrenia (OR 2.28, 95% CI 2.10–2.47), even after adjusting for SES, parental suicidal behavior and offspring mental illness (OR 1.68, 95% CI 1.53–1.85). These

results were consistent across offspring age and parental gender. In addition, separate analyses for completed and attempted suicide gave similar results.

Table 5. Odds ratio of suicidal behavior in offspring of parents with schizophrenia by attempted and completed suicide

	Number of pairs		Odds ratio (95% CI)	
	Cases	Controls	Crude	Adjusted ^a
Any suicidal behavior	119 092	568 104	2.28 (2.10-2.47)	1.68 (1.53-1.85)
Attempted suicide	108 366	517 336	2.27 (2.09-2.48)	1.67 (1.51-1.84)
Completed suicide	13 521	64 136	2.54 (2.03-3.18)	2.18(1.68-2.82)

^aRisk estimates were adjusted for education level, parental suicide and offspring mental illness

Cousin comparisons

We identified 4285 pairs of full-siblings and 662 pairs of half-siblings discordant for schizophrenia. We found a statistically significant increased risk of suicidal behavior among both full-siblings (OR 1.96, 95% CI 1.66–2.31) and half-siblings (OR 1.69, 95% CI 1.17–2.44). The effect size was slightly lower than the offspring suicide risk within the general population (depicted in Figure 8). These results suggests that the association between parental schizophrenia and offspring suicidal behavior remains, even after controlling for familial (genetic or shared environmental) confounding.

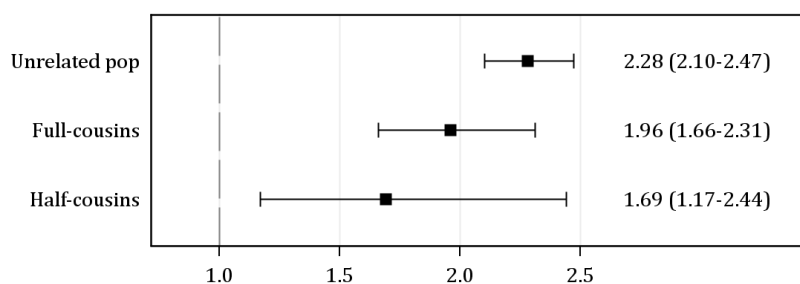


Figure 8. Odds ratios and 95% confidence interval of suicidal behavior among cousins differentially exposed for parental schizophrenia

7.4 PAPER IV

Characteristics of the participants

Among all ADHD probands 17 309 (33%) had a comorbid disorder of substance use disorder, depression, anxiety, conduct disorder, bipolar disorder, schizophrenia and antisocial personality disorder. The most common co-occurring disorder with ADHD was substance use disorder (14.1%) closely followed by depression (13.3%). The percentage of probands with attempted suicide was 9.4% compared to 1.3% of the controls, while the percentage of all ADHD probands with a co-occurring diagnose of completed suicide was 0.2% compared to 0.02% of the controls.

Main findings and family based design

We found an increased risk of attempted suicide (OR=8.45; 8.07-8.87) and completed suicide (OR=12.22; 8.67-17.22) among individuals with ADHD. The observed risk estimates for attempted and completed suicide were highest among first-degree relatives of ADHD probands and lower for more genetically distant relatives (Figure 9). For example, the risk of attempted suicide among first-degree relatives of ADHD probands, such as parents and full-siblings, was 2.42 (95% CI=2.36-2.49) and 2.28 (95% CI=2.17-2.40) respectively. This risk was considerably lower among secondary- and third-degree relatives (half-siblings: ORs between 1.57 and 1.59; cousins: OR=1.39).

Findings from the family-based analyses are depicted in Figure 9. The results of first-degree relatives being more likely to attempt suicide than second-degree and third-degree relatives indicate that genetic mechanisms are important for the familial aggregation of ADHD and suicidal behavior; based on first-degree relatives share 50% of their segregating genes while second-degree and third-degree relatives only share 25% and 12.5% respectively. In addition, the risk estimates for maternal half-siblings and paternal half-siblings were similar, indicating low support for shared environmental influences.

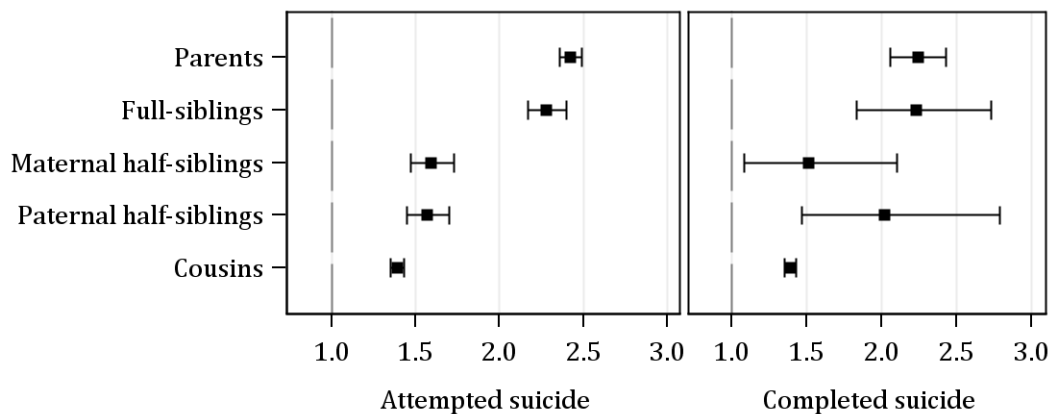


Figure 9. Odds ratios with 95% CI for attempted and completed suicide among relatives of ADHD probands

Sensitivity analyses

We also tested the importance of shared etiological factors by applying two sensitivity analyses. First, we investigated the risk of attempted and completed suicide among relatives after excluding individuals with any lifetime comorbidity of suicidal behavior and ADHD in both probands and relatives. Second, results from analyses excluding substance use disorder, depression, anxiety, conduct disorder, bipolar disorder, schizophrenia and antisocial personality disorder still indicated an increased risk of suicidal behavior among relatives with ADHD. The familial risks were slightly lower, but the same pattern was observed across family relationships confirming the importance of shared genetic factors for this association.

8 DISCUSSION

8.1 FINDINGS AND IMPLICATIONS

In this thesis we have found support for bidirectional effects between parental characteristics and offspring internalizing behaviors. Specifically, Internalizing behaviors in offspring predicted both parenting and parental psychiatric disorders. However, we could also show that specific parental psychiatric disorders predicted offspring internalizing behaviors. Some of these associations were primarily explained by a shared genetic liability for psychiatric disorders, whereas other associations were suggested to at least partly reflect environmental mechanisms. We could also show that internalizing behavior to some extent shared genetic factors with ADHD.

8.1.1 Parenting and offspring internalizing behavior

In Paper I we explored the association between parental EOI and internalizing behavior and found that internalizing behavior among adolescent girls predicted increased levels of EOI among their parents three years later, even after controlling for the stability in parental EOI. In contrast, no directional effect was found for the association between parental criticism and internalizing behavior.

We could also see that the observed child-driven effect was almost entirely explained by genetic factors. These results might be interpreted as evocative gene-environment correlation. That is, daughters genotype evoke an emotional overinvolved behavior from their parents. However, we could not rule out that the offspring's genotypes might be correlated with the family environment provided by their parents. If so, part of the association might reflect passive rGE.

This study was conducted during late adolescence which indicate that the observed child-driven effect might be specific to this time period. This is important given the variability across studies. For example, a recent finding indicated that sensitive parenting in childhood predicted reduced levels of internalizing problems in adolescence [120]. Another study found bidirectional effects between parental warmth and depressive mood based on a childhood sample [15]. Thus, important shifts in the child-driven and parent-driven effect seem to occur across the development. Moreover, previous findings from the THCAD sample have suggested that the genetic factors involved in anxiety and depressive behavior in childhood are different from adolescence. Specifically, genetic factors that were important during childhood diminished throughout development whereas new genetic factors emerged during adolescence. These findings suggests that internalizing behavior is a complex disorder with genetic innovation and attenuation across the life span [121]. In order to increase the understanding of the association between parenting and internalizing behavior, future research might benefit from studying the dimensions of internalizing behavior separately, such as withdrawn behavior and anxious-depressed behavior which represents shyness/detached behavior and sadness respectively [77, 122].

When studying associations longitudinally, one could criticize that we may actually measure two different constructs of internalizing behavior over time. Specifically, at age 16-17 the twins completed the Youth Self Report and parents answered the Child Behavior Checklist whereas at age 19-20 the Adult Self Report and the Adult Behavior Checklist were used to capture internalizing behavior among young adults. These questionnaires are very similar but include some specific questions for each time point. To increase the likelihood of measuring the same phenotype over time we only included items in common for both time points. We checked the reliability of the reduced scales with Cronbach's alpha which showed acceptable consistency across time.

Taken together, findings from this study suggested that genetically influenced child-driven effects underlie the parenting-internalizing association, and clarify that the role of such effects may differ depending on sex, type of parenting and developmental period. Research focusing on different directional effects in the parent-child relationship are important to improve our understanding of the mechanisms involved and helps in refining treatment and intervention efforts. In addition, the large residual variance of parental EOI at follow-up indicates that part of the phenotypic variation remains unexplained. Thus, other environmental factors that account for an emotional overinvolved behavior among parents also need to be explored. In line with a recent review, our findings indicate that specific parenting may actually reflect child internalizing behavior. Thus, research focusing on different rGE is crucial to better understand the development of internalizing problems in offspring [36].

8.1.2 Parental psychiatric morbidity and offspring death

In Paper II we investigated if psychiatric morbidity among parents bereaved of a child was related to the cause of death, and if such a link was consistent with a causal explanation. There are two main findings of the present study.

First, our data suggested that the increased risk of psychiatric hospitalization among bereaved parents was almost entirely confined to parents whose offspring died from suicide. These results are consistent with previous research, which have found that parents bereaved by suicide have higher levels of depression [123] and severer grief reactions compared to other causes of death [124-126]. However, they disagree with a systematic review that found no differences regarding psychiatric morbidity between individuals, albeit not specifically parents, bereaved from suicide and those bereaved from other causes of death [65]. This might be explained by limitations of previous research such as low response rates and small sample sizes [65].

Second, results from the family-based analyses indicated that the increased risk of parental psychiatric ill-health among parents who lost their offspring through suicide was most likely explained by a shared genetic liability for psychiatric disorders. These results are mainly based on analyses using stepfamilies, where no increased risk of psychiatric hospitalization was observed among stepfathers

exposed to the death of their stepchild. Because most children stay with their biological mother upon parental separation, stepfathers would share the same family environment with their stepchildren but have no biological link which could be interpreted against a strong causal relationship. The additional sibling comparison analysis was rather a complement to the stepfamily analyses, since these analyses could have different interpretations. Importantly, the results from these family-based analyses are not proof of non-causal relationships, but rather an indication of that the association between offspring death and parental psychiatric disorder is at least partly due to familial confounding. This interpretation is in line with several family and adoption studies indicating that familial transmission of suicidal behavior is partly, but not entirely, attributable to genetic factors [27, 79]. Furthermore, as individuals with a history of psychiatric morbidity have higher rates of suicidal behavior [25, 127-130], a shared genetic liability for psychiatric disorder are likely to explain most of the increased risk of psychiatric hospitalization among parents exposed to offspring suicide.

Overall, our results indicate that most of the association between offspring premature death and subsequent parental psychiatric morbidity reflects offspring suicide. Although offspring suicide is likely to increase psychiatric ill-health and contact with mental health services among affected parents, no direct causal mechanism from offspring suicide to parental psychiatric hospitalization was supported by our data. Regardless of the mechanism, the risk increase motivates extended psychosocial support and clinical attention for parents whose offspring committed suicide compared to those who lost a child from other causes.

8.1.3 Parental schizophrenia and offspring suicide risk

Paper III was the first population-based study to investigate the risk of suicidal behavior from early adolescence through young adulthood among offspring of parents with schizophrenia. Our results showed that offspring of parents with schizophrenia had a two-fold increased risk of both attempted and completed suicide regardless of time period and parental gender. In addition, these findings extend the current knowledge by indicating that part of the association is due to environmental mechanisms related to having a schizophrenic parent. In line with these findings, several earlier studies have indicated that environmental factors such as the quality of the parent-child relationship [86], lack of parental communication or support [86], and maladaptive parenting and child abuse [87] are important risk factors for suicidal behavior in offspring. Moreover, an increased suicide risk has also been observed in offspring the more recently their parent was admitted for an psychiatric disorder [131], which further support our findings that offspring suicidal behavior is likely to be influenced by parental schizophrenia through emotional and environmental effects than via a shared genetic basis only.

We further adjusted for selected mediating factors to understand if the association between parental schizophrenia and offspring suicidal behavior was entirely explained by these influences. One specific concern might be that removing all offspring with mental illness could underestimate the risk if there is a genetic link

between parental schizophrenia and offspring suicidal behavior. However, we only included offspring between 12 and 30 years of age which means that they have probably not passed through the age for risk of many mental disorders, which indicates that adjusting for offspring mental illness might not have a large effect.

There should also be noted that we did observed 63 parent-child relationships where the parents were diagnosed with schizophrenia after their offspring's suicidal behavior which might indicate influences of reversed causality (i.e. offspring suicidal behavior predict hospitalization of schizophrenia among their parents). However, schizophrenia is a major chronic mental disorder that severely afflicts the individuals with this disorder and the rearing environment for children over prolonged periods compared with other psychiatric disorders [132, 133]. By definition, schizophrenia has to be preceded by prodromal symptoms such as delusions, mood symptoms, and hallucinations for at least six months before diagnosis. Thus the course of schizophrenia is highly variable and an exact onset of schizophrenia is often not possible [134]. Therefore, the few parents identified with schizophrenia after the suicide event of their offspring were most likely having similar behavioral problems as parents diagnosed before such event. Although these 63 parents were included in the analyses we did also perform sensitivity analyses where we excluded these individuals and the results were consistent. Thus, our findings are probably not be affected by reversed causality.

To conclude, Paper III suggested that parental schizophrenia increase the risk of suicidal behavior in offspring through, at least partly, environmental mechanisms (e.g. adverse upbringing). These findings should inspire increased attention to suicidal ideation and prevention efforts in adolescent and adult offspring of parents with schizophrenia. Further research is needed to delineate what environmental risk factors and mechanisms should be targeted with appropriate psycho-educational, psychotherapeutic or even pharmacological interventions to decrease this risk of suicidal behavior in offspring.

8.1.4 Genetic overlap between suicidal behavior and ADHD

In Paper IV we estimated whether ADHD and suicidal behavior share genetic and environmental risk factors. ADHD is among the most heritable phenotypes and have recently been found to be associated with an increased risk of suicidal behaviors [17, 135, 136]. This research question extends the findings from Paper II by more specifically investigate the genetic mechanisms that explain the association between offspring suicidal behavior and parental psychiatric hospitalization.

To our knowledge, this is the first study to show that ADHD and suicidal behavior share genetic risk factors. One potential explanation to these results are pleiotropic genetic effects [137] for ADHD and suicidal behavior, possible reflecting genetic variants associated with impulsivity; a trait dimension that is highly heritable [138], a core component of ADHD and strongly associated with suicidal behavior [135, 139].

These findings also contribute to at least three novel results. First, we could show that the observed familial aggregation pattern remained when tested individually for attempted and completed suicide. Most previous research exploring the association between ADHD and suicidal behaviors have used small clinical samples and focused primarily on completed suicide [26, 136].

Second, we could demonstrate that the familial risk of suicidal behavior remained similar even after excluding relatives with an ADHD diagnosis (i.e. never been medicated for symptoms of ADHD). This is important given that several studies, including case-reports, have shown significantly higher rates of suicidal behavior among ADHD patients treated with atomoxetine, an effective treatment for ADHD used worldwide [140-142]. However, the observed familial aggregation pattern in our study indicate that ADHD medication is unlikely a plausible explanation.

Third, our results were not explained by coexisting psychiatric disorders. Even after rigorously adjusting for a broad set of psychiatric disorders in common for ADHD and suicidal behavior there was still a similar familial aggregation pattern. These findings do not rule out pleiotropic effects, but it suggests that at least part of the genetic overlap is specific for ADHD and suicidal behavior. However there are some conflicting results indicating that ADHD are strongly associated with attempted suicide only in the presence of anxiety disorders, bipolar disorders, conduct disorder, oppositional defiant disorder or substance use disorders [16]. However these results are probably due to small sample size which makes it difficult to detect a significant association.

This is an important first step towards identifying the underlying mechanisms for the risk of suicidal behavior in ADHD patients and suggests that individuals with ADHD and their family members represent important targets for suicidal prevention and treatment.

8.2 METHODOLOGICAL CONSIDERATIONS

8.2.1 Internal validity

The results from the association between exposure and outcome could be influenced by two broad categories of errors; systematic errors (i.e. selection bias, information bias and confounding) and random errors (i.e. caused by chance). Systematic errors can be handled by the investigator through adequate study design and adjustments in the analyses. Random errors could not be corrected for but are decreased with increasing sample size. High internal validity is defined as lack of systematic and random errors.

8.2.1.1 Selection bias

The results from epidemiological studies are influenced by selection bias when the distribution of exposure in controls is different from the distribution of exposure in the population. Selection biases can occur in the design stage and are generally

more common in a case-control setting. For example, case-control studies could have inappropriate selection of controls and cohort studies might have selective loss to follow-up [143]. These types of biases can be handled by making sure of that the exposure distribution in the controls and in the population is comparable. However, there are selection biases that could occur irrespectively of study design, such as missing data, which should be handled in the analyses when possible.

In Paper I we followed a population-based sample of twins over time. Although we included all Swedish twins born between May 1985 and December 1986, selective attrition might have led to somewhat biased estimates. We found slightly higher levels of parental EOI and criticism among responders compared to non-responders. In contrast, the levels of internalizing behavior were somewhat lower among responders compared to non-responders. Because the selection of offspring and parents who participated in the study is inversed, the findings from Paper I are most likely not effected of selection bias.

In Paper II we used a cohort based on the multi-generation register which included all parents and offspring in Sweden since 1932. Thus, we have no reason to believe selection bias due to loss of follow-up.

In Paper III and IV we used the nationwide registers to randomly select controls, which indicate that the controls are representable to the entire population. However, the use of register data might include some problems regarding left truncation (lack of information before register start) and right censoring (inability to follow individuals after the end of register follow-up). We handled these limitations by matching on birth year to ensure that cases and controls had equivalent time at risk to enter registers and equal time at register follow-up. Thus, if any, the level of selection bias is considered to be minimal.

8.2.1.2 Information bias

Information biases, or misclassification, arise when the information related to exposure or outcome is erroneously measured and might lead to people being classified into incorrect categories [105]. Misclassification is often mentioned in the context of sensitivity and specificity. Sensitivity is the proportion of individuals with the outcome that is correctly classified as having the condition, while specificity refers to the proportion of healthy individuals that are correctly classified as healthy. Misclassifications of outcome and exposure could lead to false associations and inconsistent findings due to reversed causality. However, this problem is uncommon in study designs where the exposure is collected prior to the outcome as in population-based nested case control studies and cohort studies used in this thesis. If the misclassification is similar among study groups it is said to be differential and non-differential otherwise. Differential misclassification could overestimate or underestimate an effect, whereas non-differential misclassification tends to dilute the effect towards the null [105].

Questionnaires and interviews are susceptible to recall bias and could occur when individuals are asked to remember information from the past. Information collected through questionnaires is also prone to underreports and over estimations. In Paper I we estimated internalizing behavior and parenting with self-reported and parent-reported questionnaires where participants were asked to remember details from the past six months. These questionnaires have been found to provide reliable and valid data [8, 94]. We also used different types of informant data to further improve our estimates; a frequently used approach, especially in child and adolescence psychopathology [8, 144]. Findings have indicated that adolescents report more problems than parent reports, and are therefore indispensable informants of their internal states [145]. However, parents are considered to be more accurate than child reports and discrepancies between adolescent reports have been shown to be smaller for internalizing problems than for externalizing problems [146], although findings have been inconsistent [147]. In Paper I, we additionally investigated potential informant biases by conducting sensitivity analyses of the observed child-driven effect in girls. The child-driven effect was still significant when using only a self-reported measure of internalizing behavior although the strength was somewhat weakened. This suggests that both offspring and parents contribute to the assessment and combining the measure might have reduced potential misclassification bias.

In Paper II-IV we used the Swedish population based registers to define suicidal behavior. Register data eliminates the risk of recall bias and reduce the risk of misclassification. However, we could not entirely exclude possible misclassification; for example completed suicide might be underestimated because of misclassification as other causes of death and suicide attempts might not be registered. Nevertheless, because we have no reason to believe misclassification of suicidal behavior to be differential (i.e. in paper II the diagnoses of suicidal behavior in offspring is not depending on whether their parents had schizophrenia or not), this misclassification, if any, could decrease the precision of the estimates but would only bias the results towards the null. To further reduce possible misclassifications, suicidal behavior was only allowed in individuals of age 12 years or older. There are examples of suicidal behavior in the registers (see Figure 10) but it is difficult to know to what extent these estimates are reliable.

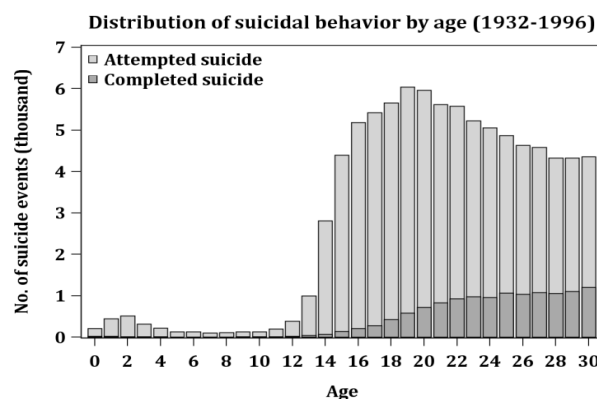


Figure 10. Distribution of suicidal behavior by age between 1932 and 1996

Psychiatric disorders were defined in Paper II-IV based on the Swedish nationwide registers. Specifically, in Paper II and III psychiatric disorders were defined on the basis of hospitalizations using the in-patient register, which limits the assessments to more severe forms of psychopathology. However, it is important to note that, inhabitants of Sweden have access to generally available tax-financed health care that usually ensures equal access to in-patient care, which indicates high sensitivity and high specificity of the severe psychiatric disorders. However, we could not entirely rule out potential differential misclassification of outcome in Paper III. Hospitalization of parents might have occurred more often if clinicians heard of their bereavement of suicide as compared to any death. Such potential bias would inflate the association between offspring suicide and parental psychiatric disorders. However, the absence of a risk increase among bereaved stepfathers and similar risks among differentially exposed siblings did not support strong bias of this type.

In Paper IV we used both the in-patient and out-patient register to define ADHD which also captures the milder forms of ADHD. Moreover, we identified ADHD patients from individuals treated with medication for ADHD. The authority to prescribe medication of ADHD in Sweden is restricted to physicians specialized on ADHD treatment, which suggests that use of medication is a valid indicator of ADHD. Likewise, a recent validation check also indicate high specificity (i.e. healthy subjects are rarely defined as cases) of ADHD diagnoses in Swedish registers [148].

8.2.1.3 Confounding

It is commonly known that the relationship between two variables could depend on the effect from a third variable. In general, an association between exposure and outcome that is due to common causes might lead to spurious associations; a bias referred to as confounding (see Figure 11a) [143]. Confounding can be handled by randomizing subjects into different exposure groups. However this design is not always feasible or ethical. Other ways to control for confounding is through matching, stratification and in regression models through adjustments.

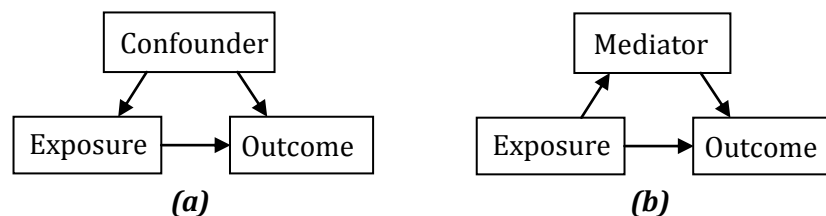


Figure 11. Direct acyclic graph illustrating confounding and mediation between exposure and outcome

Matching can prevent confounding in cohort designs, but in a case-control setting matching has to be taken into account in the analyses [105] (for more details see section 5.1). Stratifying on potential variables removes the confounding in these strata. In addition, adjusting for several confounders could be made in regression

analyses, which means that we estimate the association within each stratum while holding all other variables constant.

A covariate which is an intermediate step in the causal pathway between exposure and outcome are known as a mediator (see Figure 11b). A mediator is not a confounder but an effect we often want to study, and adjusting for such variables might introduce bias of unmeasured confounding between the mediator and the outcome. However, in observational studies we can never entirely exclude all potential confounding because we might have unmeasured or misclassified confounders.

In Paper II-IV we used the population based registers to find information of several important confounders in the association between suicidal behavior and psychiatric illness. In Paper II we expected the effect of the loss of a child on parental psychiatric disorders to be affected by SES, parental gender, parental age, offspring age and number of children. We assessed SES by highest attained education level which has been very stable over time compared to employment, civil status and income. However, the education level has gradually increased in the population which could introduce bias across age groups. The case-control design used in Paper II was matched on birth year and gender which should minimize this type of bias. We stratified on parental gender, and offspring age to get separate effect estimates in these categories.

In addition to potential confounders, in Paper III we were also interested in whether the effect of schizophrenia on offspring suicide was entirely explained by parental suicidal behavior and offspring mental illness. Therefore, we assessed the contribution of these mediating factors by individual adjustment.

8.2.1.4 Random errors

Variability in the data that cannot be explained by systematic errors is called random errors. To exclude that an association was a chance finding the risk estimate is often followed by a confidence interval or a p-value of less than 0.05 which indicates that the estimate has 95% probability of being within the range of the confidence interval. However, this is correct given that there are no systematic errors. The papers in this thesis are based on large population-based samples and the amount of random errors is most likely minimized. Nevertheless, the statistical power was sometimes limited because of the rare exposure of completed suicide and these results should be carefully interpreted.

8.2.2 External validity

External validity refers to how well the results are generalizable to other populations and time intervals. Nevertheless, a requirement for external validity is acceptable internal validity.

The studies in this thesis are based on samples from the nationwide registers during the past decades and should be justly transferable to the Swedish population and similar nationalities. However, we experienced some attrition in

Paper I due to follow-up which might have reduced the generalizability of those results to individuals with milder forms of internalizing problems. Nevertheless, generalizations to other more ethnically heterogeneous populations than Sweden should be made with caution.

8.2.3 Assumptions of the twin design

8.2.3.1 The equal environment assumption

One of the main assumptions of the classical twin design is the equal environment assumption (EEA). It implies that the shared environment (of importance to a phenotype) is similar for twins reared in the same family [74]. The EEA is violated if MZ twins are treated more similar than DZ twins, an implication that could overestimate genetic influences and underestimate shared environmental effects. Although the validity of the EEA have been debated [150], it has been supported in twin studies of psychiatric disorders [151, 152] and parental behaviors [153, 154]

8.2.3.2 Assortative mating

The twin method also rest on the assumption of random mating. Assortative mating occurs when people tend to choose partners that are more similar with regards to a specific phenotype. As a consequence DZ twins could get a higher genetic correlation than 0.5 which would overestimate the shared environmental effect. The focus of Paper I was on parental behavior instead of psychiatric disorders. Thus, the level of possible internalizing problems among parents was unknown. However, the literature has reported assortative mating for depressive disorders [155], which indicate that the shared environmental influences might be somewhat overestimated.

8.2.3.3 Generalizability of twins

The results from the twin model are only valid if twins are representative to the general population. Although twins are different to singletons in some aspects (e.g. birth weight and obstetric complications), they are not differences with regards to the prevalence of childhood internalizing disorders [156]. In addition, findings from the Swedish twin register indicated that the incidence if affective disorders did not differ from the general population [152]. Thus, the results of Paper I should be generalizable to the singleton population. However, the attrition at follow-up should be taken into account when considering the generalizability of the results.

8.2.3.4 Gene-environment interplay

Another assumption of the twin model is the independent influence of genes and environments. However, an observed association might be due to correlation or interaction between genes and environment [68]. Thus, a part of a genetically influenced effect might reflect passive gene-environment correlation (rGE), which means that the children's genotypes are correlated with the family environment provided by their parents [74]. If passive rGE is present the influence of shared environment effects tend to be increased [157]. In addition, individuals' genetic sensitivity to different environments (in contrast to genes and environment

individually) might also contribute to mental health problems, an effect known as gene-environment interaction (GxE). A positive GxE will result in a larger non-shared environmental component.

In Paper I, it was not possible to distinguish between evocative rGE and passive rGE. In addition, high parental EOI might only affect children who are genetically vulnerable for such environments. This suggests that both rGE and GxE could co-occur in the development of adolescent internalizing problems. However, since these mechanisms tend to underestimate the genetic effect, our findings of a genetically influenced child-driven effect underlying the parenting-internalizing association are most likely robust.

8.2.4 Assumption of other family based designs

We have used several different quantitative genetic designs to control for genetic and putative environmental risk factors shared by related individuals. However, each family-design is based on specific assumptions which should be taken into account when interpreting the results.

In general all family based designs require large samples to detect associations and control for confounding. Only exposure discordant relatives are informative in quantitative genetic designs and a large sample is therefore needed to detect such relative-pairs. This is particularly important if genetic factors are central for the association, which would make it even more difficult to find exposure discordant relatives. In additions, large samples are also needed to increase power for rare exposures and outcomes. Even though we used large samples with data from population based registers, the statistical power were limited because of rare events like suicidal behavior and schizophrenia.

A note should also be taken on the reliability of fatherhood in the registers. The husband of the mother is seen as the biological father of the offspring even if the mother was recently widowed. Paternity is otherwise reported by the mother, or in some cases, by a court. The paternal discrepancy in Swedish register has not been reported but other reports indicate a median discrepancy of 3.7% [158]. A possible misclassification mean that some relatives would be more distantly genetically related (i.e. detected siblings would in reality be half siblings) which means that our estimated familial risks between parents and offspring might be somewhat underestimated, whereas sibling comparisons slightly overestimated.

The stepfamily design used in Paper II was based on two main assumptions. First, children were assumed to stay with their biological mother upon parental separation. This pattern has been documented by Statistics Sweden and refers to the majority of children among divorced parents [72]. Second, stepfathers are also assumed to share the same family environment and be equally emotionally involved in their stepchildren as the biological mother is. However, stepfathers could be introduced in the family quite late in the children's development which might reduce the level of attachment. The lack of psychiatric disorders in bereaved stepfathers might therefore not be interpreted as the absence of a causal

effect, but instead explained by a low attachment to their stepchildren. However, we tried to address this assumption by examining the association among step fathers involved since the step child was at least 5 and 7 years old. We found the same results, which strengthens the interpretation of a non-causal effect for this association.

The sibling comparison design applied in Paper II was also based on some important assumptions. First, the exposed sibling is assumed to not influence their unexposed sibling [71]. Also, siblings are assumed to be generalizable to singletons [66]. Previous studies have generally used sibling comparisons to test for causal inferences of siblings exposed to different maternal characteristics, for example smoking during pregnancies [159-161]. However, in Paper II we use the sibling comparison to test for causal inferences regarding siblings exposed to different offspring characteristics, for example suicidal behavior. In this setting, unexposed siblings are also likely to be influenced by the death of their niece/nephew, and the results could therefore be interpreted as both a direct causal mechanism as well as familial confounding. Because this analysis could not be interpreted by itself, we had to use the sibling comparison analysis as a complement to the stepfamily design. The stepfamily analyses in Paper II indicated that the association between offspring suicidal behavior and parental psychiatric disorders was explained by a shared genetic liability for psychiatric disorder rather than causal mechanisms, thus we could interpret the sibling comparison results based on the suggestion that there is no strong causal effect between offspring suicidal behavior their aunt/uncle. Although the sibling comparison design could control for unmeasured familial confounding shared between siblings, we cannot entirely exclude bias from non-shared environment and measurement errors [149], which indicate that our findings should be interpreted with some caution.

The cousin comparison design used in Paper III assumes that cousins are generalizable to other family structures. Further, we assume that the cousins share the home environment with their nuclear family to a large extent. Intuitively, the effect of parental schizophrenia on offspring suicide risk might be affected by how long they have lived together. To increase the probability that offspring had recently lived together with their parents, age 30 was chosen as a cut-off. We also tested different periods of life and found that offspring suicide risk related to parental schizophrenia did not vary according to age.

Lastly, in Paper IV we used a combination of different genetically informative designs (including sibling-comparisons and cousin comparisons as mentioned above) to study the risk pattern for the association between ADHD and suicidal behavior. One additional major assumption was made to study the importance of shared environmental influences; that the family environment is assumed to be shared between maternal half-siblings but non-shared among paternal half-siblings. This relates to the previously mentioned fact that offspring predominantly lives with their mothers when parents separate. If paternal half-siblings share environment to a larger extent this comparison would not be

informative. The major advantage of the family-based design in Paper IV is the combination of several quantitative genetic designs.

Still, the results from a particular quantitative genetic design are not proof of non-causal relationship. However, if several different genetically informative designs indicate the same results we can more confidently suggest, or reject, a causal interpretation. Thus, combining different genetically informative designs is important to more thoroughly examine causal inferences.

8.3 CONCLUDING REMARKS

This thesis has demonstrated the role of genetic and environmental factors in the association between different parental characteristics and offspring internalizing behavior. Our findings that the development of internalizing behavior could partly be explained by genetic factors do not mean that these levels are unchangeable. In fact, recent findings confirm that internalizing behavior is a complex disorder with genetic innovation and attenuation across the life span [121].

Our familiar clustering results provide important targets for prevention and treatment. For example, the increased risk of both attempted and completed suicide in relatives to ADHD patients suggests that family members of individuals with ADHD should be screened for suicidal behaviors given that untreated suicide attempts may lead to completed suicide and substantial adversity in the family environment.

The results regarding bi-directional effects have also implications for interventions. Specifically, parents with schizophrenia were associated with an increased risk for suicidal behavior among their offspring; an association suggested to be at least partly due to environmental mechanisms. These results indicate that environmental factors such as inadequate parental care, maladaptive parenting and child abuse might influence suicidal behavior in offspring. Because schizophrenia is a major chronic mental disorder that severely afflicts the rearing environment for children over prolonged periods, support and interventions aimed at parents with schizophrenia should start as early as possible.

In addition, internalizing behavior in children does also elicit an emotional overinvolved behavior from their parents which indicates that potential treatment interventions could benefit from being directed towards the children during late adolescence whereas it might be more important to focus on parenting in childhood to reduce offspring internalizing behavior later in life. Thus, possible interventions include several different treatment approaches, but support the integration of services for both children and parents [162].

The results from this thesis highlight the importance of genetic confounding. These results have important implications for future gene-environment interaction (GxE) research. Previous research has indicated that interactions between specific genetic risk variants and stressful life events and maltreatment

increase the risk for depression [163]. A major limitation of this previous research is that it has failed to control for rGE [164]. Because both parenting and stressful life events have been shown to be in part influenced by genetic factors, such findings might in fact represent an interaction between genes of depression and genes influencing the environmental exposure.

Adoption studies is a powerful design that can rule out the effect of passive rGE from an association, because adoptive parents who provide the rearing environment do not share genetic factors with their adopted children, although these studies have been difficult to conduct. Recent developments in quantitative genetics have also made it possible to study GxE while accounting for rGE in the model [165]. A recent study using this approach could show that both rGE and GxE can co-occur in the development of adolescent depressive symptoms and parental punitive discipline [11]. The genetic predisposition of internalizing behavior among children could also vary across different environmental exposures, such as parental divorce [166]. The interaction between genotypes and specific environments promotes interventions aimed at improving the children's environment in order to reduce internalizing behaviors among offspring.

Quantitative genetic designs have become an essential tool to investigate if environmental exposures are truly purely environmental, but also to give strong indications for which phenotypes that may be important for molecular genetic studies [68]. The findings of this thesis are only an initial step towards identifying the underlying mechanisms for internalizing behavior in offspring and parental characteristics. There is still a need to further understand the complex nature of internalizing behavior problems among children, and especially how these problems associate with parental characteristics. Observational studies could not by themselves provide proofs of causation, and randomizing children to experimental adverse environments are not possible. Thus, genetically informative designs will continue to provide important insight to the etiological basis of parental characteristics and offspring internalizing disorders in future research.

9 CONCLUSIONS

- I Parental emotional overinvolvement stems in part from daughters genetic predisposition for internalizing behavior. The strength of the child-driven effect may differ depending on offspring gender, type of parenting and developmental period.
- II Parental psychiatric morbidity following offspring death was primarily found among parents exposed to offspring suicide. This association was mostly explained by a shared genetic liability for psychiatric disorder rather than a causal environmental mechanism judging from family-based analyses.
- III Parental schizophrenia was associated with a two-fold increase risk of suicidal behavior in their offspring. These results were consistent across different periods of life and independent of parental gender. Family-based analyses indicated that the increased risk of suicide in offspring was at least partly due to environmental mechanisms related to having a schizophrenic parent.
- IV ADHD was strongly associated with attempted and completed suicide. An increased risk of both completed and attempted suicide was also found among relatives of individuals with ADHD. The pattern of familial aggregation suggested the importance of shared genetic factors these associations.

10 SVENSK SAMMANFATTNING

Internaliserande problem så som depression och självmordsbeteenden är vanligt förekommande psykiska problem som orsakar stora kostnader för samhället i form av slutenvård och skador, men även lidande för individen i fråga och dess omgivning. Under de senaste åren har forskning visat att både arv och miljö är av betydelse för utveckling av internaliserande problem hos barn och ungdomar. Ett väldigt viktigt nästa steg inom denna forskningslinje är att förstå hur samspelet mellan arv och miljö påverkar individens risk att utveckla mental ohälsa och psykisk sjukdom. Sådan kunskap kan sedan användas för att utveckla effektiva förebyggande åtgärder och för att identifiera barn med dålig prognos som behöver extra hjälp för att lyckas bättre i framtiden. I denna avhandling har vi undersökt riktningen och etiologin bakom föräldrars egenskaper och deras barns internaliserande beteenden med hjälp av olika genetiskt informativa modeller. Studierna baseras på data från stora svenska populationsbaserade register som gör det möjligt att undersöka ovanliga beteenden så som självmord samt att identifiera familjekonstellationer som syskon, kusiner, föräldrar och barn.

I första studien fann vi att internaliserande beteenden hos döttrar framkallade ett överbeskyddande beteende från sina föräldrar. Tvillingmodeller visade att associationen främst förklarades av genetiska faktorer.

Den andra studien visade att föräldrar som upplevt självmordsbeteenden hos sina barn hade betydligt högre risk för efterföljande psykiatrisk sjukdom. Familjebaserade analyser visade att en genetisk ärftlighet för psykiatriska sjukdomar förklarade en stor del av sambandet.

I den tredje studien visade vi att barn till schizofrena föräldrar hade en dubbelt så hög risk för självmordsbeteenden jämför med barn som inte hade sjuka föräldrar. Med hjälp av kusinjämförelser kunde vi visa att den ökade risken för självmordsbeteenden hos barn var associerat till en dålig uppväxtmiljö relaterade till att ha en schizofren förälder.

Slutligen fann vi en ökad risk för självmordsbeteenden hos individer med ADHD. Vi hittade även en ökad risk för självmord bland anhöriga till personer med ADHD. Familjär aggregering visade på ett genetiskt överlapp mellan självmordsbeteenden och ADHD.

Sammanfattningsvis har vi visat att internaliserande beteenden hos barn framkallade både föräldraskap och psykiska sjukdomar hos föräldrarna genom genetiska mekanismer, men vi kunde också visa att psykiatriska sjukdomar hos föräldrar påverkade internaliserande beteenden avkomman genom miljömässiga mekanismer. Dessutom kunde vi påvisa ett genetiskt överlapp mellan ADHD och internaliserande beteenden. Genetiskt informativa metoder är fortsatt viktiga för att i framtiden undersöka hur gener och miljöfaktorer bidrar till sambandet mellan föräldrars egenskaper och internaliserande problem hos deras barn.

“Learn from yesterday, live for today, hope for tomorrow. The important thing is to not stop questioning.”

— Albert Einstein

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