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PATERNAL AGE AS A RISK FACTOR FOR SCHIZOPHRENIA IN OFFSPRING

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Paternal Age as a Risk Factor for Schizophrenia in Offspring

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ABSTRACT

Schizophrenia is one of the most disabling conditions that affect human beings. There is robust evidence of an association between advancing paternal age and schizophrenia in offspring. The understanding of this association is limited, i.e. the mechanism is not known. This thesis aimed to further understand the mechanisms behind the association. The studies were conducted using Swedish register data from different sources. Different explanations were examined with possible confounders taken into account.

The first study showed that adoptive paternal age was not associated with increased risk of schizophrenia in adopted subjects. This indicates that there is no increased risk of developing schizophrenia due to the psychosocial environment of an older father.

The second study found that advancing paternal age is associated with increased risk of autism spectrum disorder in offspring. In contrast to schizophrenia this association is interacting with maternal age and advancing maternal age is associated with increased risk of autism spectrum disorder.

In the third study factors associated with both delayed fatherhood and increased risk of schizophrenia in offspring was implicated as explanation to the paternal age effect. For the second time delayed fatherhood rather than advancing paternal age per se was shown to be associated with increased risk of schizophrenia in offspring.

The fourth study found that personal characteristics could be implicated as factors explaining the association between age at fatherhood and schizophrenia.

In conclusion, this thesis provides knowledge about the association between advancing paternal age and schizophrenia. It shows that advancing paternal age is not a specific risk factor to schizophrenia even though there might be different mechanisms behind the association to autism spectrum disorder. The association between advancing paternal age and increased risk of schizophrenia in offspring is most likely due to factors that are associated with both delayed fatherhood and increased risk of schizophrenia in offspring. These factors could be related to social functioning. This has important implications regarding clinical understanding and public health advice as well as directions of future research.

LIST OF SCIENTIFIC PAPERS

I. **Ek** M, Wicks S, Magnusson C, Dalman C.
Adoptive paternal age and risk of psychosis in adoptees: a register based cohort study.
PLoS One. 2012;7(10):e47334.

II. Idring S, Magnusson C, Lundberg M, **Ek** M, Rai D, Svensson AC, Dalman C, Karlsson H, Lee BK.
Parental age and the risk of autism spectrum disorders: findings from a Swedish population-based cohort.
Int J Epidemiol. 2014;43(1):107-15.

III. **Ek** M, Wicks S, Svensson AC, Idring S, Dalman C.
Advancing Paternal Age and Schizophrenia: The Impact of Delayed Fatherhood.
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IV. **Ek** M, Wicks S, Svensson AC, Dal H, Zammit S, Hemmingsson T, Dalman C.
Personal Characteristics previously shown to be associated with Schizophrenia and their association with Teenage and Delayed Fatherhood
Manuscript

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LIST OF ABBREVIATIONS

APA	Advancing Paternal Age
ASD	Autism Spectrum Disorder
CI	Confidence Interval
CNVs	Copy Number Variations
CRC	The Conscript Register Cohort
GAM	Generalized Additive Model
GWAS	Genome-Wide Association Studies
HC	The Swedish Population and Housing Censuses
HR	Hazard Ratio
MGR	The Multi-Generation Register
NPR	National Patient Register
OR	Odds Ratio
PAR	Population Attributable Risk
RTP	The Register of Total Population
SYC	The Stockholm Youth Cohort

1 INTRODUCTION

Schizophrenia is a highly familial mental disorder with advancing paternal age as a risk factor. This thesis sets out to further understand by what mechanism advancing paternal age is associated with schizophrenia in offspring.

People with brain dysfunction have most likely existed since prehistoric time. Considering the complexity of the brain it's amazing that it functions as well as it does in most cases. Every once in a while the brain doesn't function in a way that is consider to be adequately functional. Conditions like intellectual disability and autistic disorder are present from birth and usually diagnosed in childhood[1, 2]. In contrast schizophrenia usually presents its symptoms for the first time in late adolescence to early adulthood[3]. These conditions lead to obvious dysfunctions in different domains of life and will be more disabling the more complex the surrounding environment is. Schizophrenia differs from autism in its high prevalence of psychotic symptoms, i.e. hallucinations and delusions. Hallucinations and delusions are the most prominent symptoms of schizophrenia but from a clinical perspective it is clear that cognitive and negative symptoms such as poor working memory, poor executive functioning, inability to sustain attention, and lack of motivation are much more disabling when it comes to interactions with the surrounding environment and ability to be employed or care for oneself [4].

1.1 A HISTORICAL ETIOLOGICAL PERSPECTIVE

The way mental disorders such as schizophrenia are perceived have a great impact on how people with the disorder are treated and cared for in society. Over time the perception has changed. It's likely that we are more informed today than ever before, but we still don't fully understand the mechanisms. This merits a humble approach in order not to create unnecessary stigma.

Early etiological considerations included the belief that mental disorders were caused by "demons" possessing the body. An early account of this belief is in the Old Testament in the First Book of Samuel. Here we can read about how God was angry with King Saul since King Saul neglecting his religious duties. To punish Saul, God tormented him with an evil spirit. This can very well be interpreted as some kind of mental disorder. The cure in this case was music.

First Book of Samuel 16:
[14] Now the Spirit of the Lord had departed from Saul, and an evil spirit from the Lord tormented him.
[15] Saul's attendants said to him, "See, an evil spirit from God is tormenting you. Let our lord command his servants here to search for someone who can play the lyre. He will play when the evil spirit from God comes on you, and you will feel better."
[23] Whenever the spirit from God came on Saul, David would take up his lyre and play. Then relief would come to Saul; he would feel better, and the evil spirit would leave him.

Hippocrates (460-377 BC) thought that mental illness was due to an imbalance between the four fluids: blood, yellow bile, black bile, and phlegm. Thus it could be treated, and perhaps cured, with diets, laxatives, and blood-lettings. This was a major shift in the way mental illness was conceived, from thinking God was the cause to believing mental illness was a change within the body that could be dealt with through affecting bodily functions.

In the middle ages there was a backlash to the religious believes, but prayers and confession was combined with laxatives and blood-lettings.

The French physician Philippe Pinel (1745-1826) theorized that mental illness was a result of psychological and social stressors. Mental disorders should thus be treated through showing respect for the person, decreasing stimuli, establishing a trusting and confiding doctor-patient relationship, routine activity, and abandonment of the Hippocratic treatments.

Schizophrenia was first described by Emile Kraepelin (1856-1926) in 1896 [5]. He was a physician from Germany who categorized mental illness into different disorders and used the term Dementia Praecox when he described what later has been called Schizophrenia. The term Schizophrenia was coined in 1911 by Eugen Bleuler (1857-1939). As most diagnostic terms it has Greek roots, "schizo" meaning split and "phrene" meaning mind.

Kraepelins description was based on neurological and behavioral abnormalities that he noted in the childhood history of his patients[5]. His findings suggest a neurodevelopmental disorder. Thomas Clouston (1840-1915), a lecturer in psychiatry at Edinburgh University, described a similar group of patients and called the condition "developmental insanity" in 1891[6]. Similar observations were made during the 20th century, e.g. Bender[7] and Watt[8]. But the Austrian psychiatrist Sigmund Freud (1856-1939), contemporary with Kraepelin and Bleuler, had a profound influence on psychiatry in the 20th century giving rise to the idea that schizophrenia was due to unconscious conflicts originating in childhood [9]. This indirectly blamed especially the mothers to the patients as responsible for the condition.

In 1972 Michael Rutter (1933-) concluded that at the time commonly used "childhood schizophrenia" was "an astonishingly heterogeneous mixture of disorders with little in common other than their severity, chronicity, and occurrence in childhood" [10]. He proposed that infantile autism would be used as a term for the condition that was distinct from schizophrenia in e.g. age at onset, failure of development rather than loss of reality sense after development is established, and a steady course in contrast to remissions and relapses. Infantile autism was no longer to be a part of the schizophrenia spectrum disorders.

The concept of schizophrenia being a neurodevelopmental disorder was revisited in 1985 by Robin Murray (1944-) with colleagues[11] and Daniel Weinberger[12] and has since then been revised and modified[13].

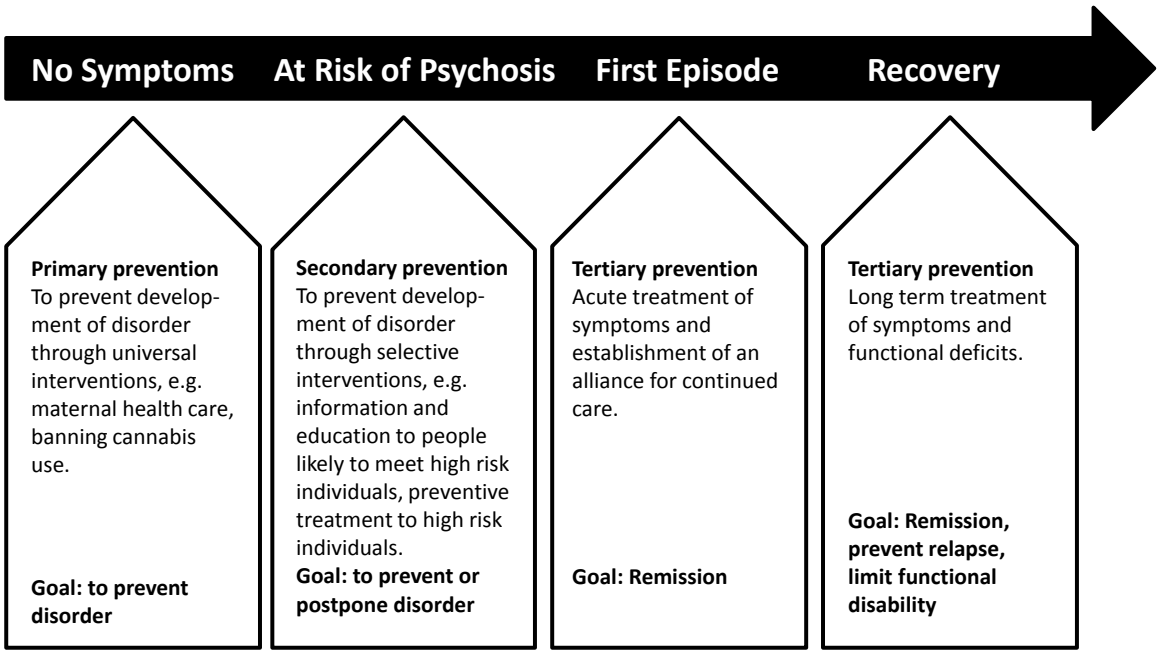
1.2 DIAGNOSTIC PERSPECTIVE

The psychiatric diagnostics are using a clusters of symptoms in the absence of known etiology, describing syndromes[14]. This is important to understand to be able to grasp the heterogeneity present within a syndrome like schizophrenia. Since the diagnosis is determined from visible or reportable function or experience it only deals with the effect of the disorder, not the underlying function. Thus it is possible that there is more than one underlying cause of a symptom. On top of that syndromes like schizophrenia may consist of variable combinations of symptoms. In brief, to fulfill the criteria of schizophrenia according to DSM-IV[15] the patient needs to exhibit at least one out of the first three symptoms and two out of the five symptoms: delusions, hallucinations, disorganized speech, disorganized or catatonic behavior, and negative symptoms. This theoretically means that people with the same diagnosis of schizophrenia could exhibit non-overlapping symptoms. It opens up for a heterogeneous disorder. However, it is also possible that one etiological factor could result in a lot of different symptomatic manifestations. There are conditions that can show the same or similar symptoms as schizophrenia, e.g. encephalitis (e.g. borreliosis), dementia, and side effects of legal or illicit drugs. In these cases there is a known cause of the disruption in behavior and thinking, a cause that does not explain the etiology for the majority of cases with schizophrenia. Thus, individuals with these conditions should not be diagnosed with schizophrenia. The most recent group of patients that are partly emerging out of patients with schizophrenia are patients with autism spectrum disorder. Sometimes their functioning is identical to patients with schizophrenia and the only thing that differs between the groups is age at onset; with autism spectrum disorders being identified from childhood while schizophrenia is identified later in life. With each new etiology that we discover the diagnosis may seem more and more specific, but we are still, most likely, dealing with a disorder that is very heterogeneous.

1.3 PUBLIC HEALTH PERSPECTIVE

The research in this thesis is focused on paternal age as a risk factor for schizophrenia, adding original information to a vast and complex body of research regarding schizophrenia. It is known since the beginning of this century that advancing paternal age increases the risk of schizophrenia in offspring. However, the etiological mechanism has remained obscure. A couple of different hypotheses has been presented that will be described in some detail in the background section. From a public health prospective it is important to understand different levels of prevention (i.e. primary, secondary and tertiary prevention) and how they may prevent schizophrenia and limit the disability burden caused by schizophrenia (Figure 1). If paternal age is considered a causal factor; primary prevention could hypothetically be a general recommendation of having children at younger age, preferably in the man's twenties. However, if advancing paternal age is not part of a causal pathway, a recommendation of early fatherhood would not only be without effect on the incidence of schizophrenia, it would be indirectly blaming older fathers to patients with schizophrenia as partly responsible (through their choices in life) for their child's disorder.

Figure 1


No Symptoms
At Risk of Psychosis
First Episode
Recovery
Primary prevention
To prevent development of disorder through universal interventions, e.g. maternal health care, banning cannabis use.
Goal: to prevent disorder
Secondary prevention
To prevent development of disorder through selective interventions, e.g. information and education to people likely to meet high risk individuals, preventive treatment to high risk individuals.
Goal: to prevent or postpone disorder
Tertiary prevention
Acute treatment of symptoms and establishment of an alliance for continued care.
Goal: Remission
Tertiary prevention
Long term treatment of symptoms and functional deficits.
Goal: Remission, prevent relapse, limit functional disability

2 BACKGROUND

2.1 SCHIZOPHRENIA

Schizophrenia is one of the most chronic and debilitating conditions to affect human beings[16]. It usually develops in adolescence or early adulthood. The measured incidence and prevalence differ between different populations and environments. In a systematic review including 1,721 prevalence estimates from 188 studies made in 46 countries all over the world the median prevalence measure was 0.33-0.46% depending on method of measuring[17]. More specifically the median values per 1,000 persons with 10% and 90% quantiles for the distributions for point, period, and lifetime prevalence were 4.6 (1.9–10.0), 3.3 (1.3–8.2), and 4.0 (1.6–12.1). The large variance in prevalence estimates in different neighborhoods and populations can be further illustrated by the range in prevalence of non-affective psychotic disorder in Stockholm County from areas with 0.35% to areas with 1.32% 1-year prevalence[18].

2.1.1 Clinical Picture

Schizophrenia is a syndrome characterized by positive and negative symptoms as well as cognitive symptoms. Depressive symptoms and substance abuse are common features in patients with schizophrenia, even though they are not part of the diagnosis per se. The positive symptoms include hallucinations (perceived input from sensory organs without external stimuli) and delusions (a "false" belief held with conviction despite evidence of the contrary). Disorganized speech and behavior can also be seen and could be considered positive symptoms. Negative symptoms are a lack of activity and include lack of motivation, drive, inability to communicate and interpret body language and facial expressions. Positive and negative symptoms can usually be observed clinically by just meeting and talking to the patient. Cognitive symptoms include concentration, working memory, executive functioning, and problem solving. These symptoms could be viewed as the underlying cause of especially the negative symptoms. They can partly be observed clinically but in general there is need to do psychological testing to get a clear picture of the patient's cognitive abilities or if you will dysfunction.

2.1.2 Contemporary Etiological Perspective

Today many researchers consider schizophrenia to be a neurodevelopmental brain disorder[19-22] for which dopamine plays a major role[23] and psychosocial risk factors are contributing to the development. Genetic variability is believed to account for most of the risk at individual level with heritability of around 80%[24], but environmental factors are in a sense of a greater interest since they possibly could be affected and are contributing with a considerable impact on population level[25], e.g. if both parents are diagnosed with schizophrenia the risk for male offspring to be diagnosed with schizophrenia is ~20% while the population attributable risk (PAR) is ~0.1% and if the male is born in an urban area his risk is ~2% while the PAR is ~12% according to a recent study on the Danish population[25].

F20 SCHIZOPHRENIA (ICD-10 criteria[26])

G1. Either at least one of the syndromes, symptoms and signs listed below under (1), or at least two of the symptoms and signs listed under (2), should be present for most of the time during an episode of psychotic illness lasting for at least one month (or at some time during most of the days).

1. At least one of the following:
    a. Thought echo, thought insertion or withdrawal, or thought broadcasting.
    b. Delusions of control, influence or passivity, clearly referred to body or limb movements or specific thoughts, actions, or sensations; delusional perception.
    c. Hallucinatory voices giving a running commentary on the patient's behaviour, or discussing him between themselves, or other types of hallucinatory voices coming from some part of the body.
    d. Persistent delusions of other kinds that are culturally inappropriate and completely impossible (e.g. being able to control the weather, or being in communication with aliens from another world).
2. or at least two of the following:
    e. e) Persistent hallucinations in any modality, when occurring every day for at least one month, when accompanied by delusions (which may be fleeting or half-formed) without clear affective content, or when accompanied by persistent over-valued ideas.
    f. f) Neologisms, breaks or interpolations in the train of thought, resulting in incoherence or irrelevant speech.
    g. g) Catatonic behaviour, such as excitement, posturing or waxy flexibility, negativism, mutism and stupor.
    h. h) "Negative" symptoms such as marked apathy, paucity of speech, and blunting or incongruity of emotional responses (it must be clear that these are not due to depression or to neuroleptic medication).

G2. Most commonly used exclusion criteria: If the patient also meets criteria for manic episode (F30) or depressive episode (F32), the criteria listed under G1.1 and G1.2 above must have been met before the disturbance of mood developed.

G3. The disorder is not attributable to organic brain disease (in the sense of F0), or to alcohol- or drug-related intoxication, dependence or withdrawal.

Several risk factors have been described and acts at different time points in the maturation of the brain: before conception[27] by genes[28-31] or environment[32], through epigenetic changes[32, 33], during pregnancy (e.g. lack of nutrients, growth restriction, pre-eclampsia) possibly affecting brain development and maturation[34-46], during delivery through asphyxia (indicated by low APGAR score)[47], and in childhood and adolescence through for example encephalitis and cannabis use [48, 49]. Even though these risk factors are likely to act negatively in the

development of the child some of them, i.e. preterm birth, low birth weight, and gestational age are also associated with severe parental mental illness[50]. During childhood and adolescence risk factors such as cognitive impairment[51], disturbances in behavior, intellectual and language deficits, motor delays and poor social functioning[52-58], alterations in language, educational performance, and physical growth[54] are more likely premorbid symptoms associated with underlying mechanisms than indication of etiological factors on their own. Although there are indications that experience of physical abuse, domestic violence and bullying increases the risk of psychotic symptoms in a population based sample[59]. It is possible that individuals with increased risk of psychotic disorders also have a higher risk of experiencing adverse events when interacting with their surroundings and that the experiences increase the risk of psychotic disorders further. A possible mechanism is through the disruption of the maturation and sensitization of the dopamine system through glutamatergic regulation of dopaminergic activity[60] as well as through changes in the cognitive schema[13]. Together with changes in brain volume before, during and after the clinical onset of the disorder[61, 62] there is strong indication that there is a subclinical brain dysfunction present prior to the clinical debut.

Thus there are indications of the presence of premorbid brain deficits that increases the risk as well as direct influence on the brain that increases the disposition to later develop schizophrenia.

There is in other words likely a combination of genetic and environmental factors that leads up to the clinical debut of schizophrenia. This could be understood according to the diathesis-stress model, i.e. early risk factors and genetic predisposition increase the vulnerability and later risk factors trigger the disorder. Once the disorder is triggered there are findings of loss in brain volume[63]. This could indicate a progressive neurodegeneration associated with psychosis, but it might also be due to pharmacological treatment, genetic factors or aging.

2.2 AUTISM SPECTRUM DISORDER (ASD)

Even though there are major differences between ASD and schizophrenia there are similarities in both the clinical picture and etiological risk factors. In a study of 46 young adults 60% of patients with clinical diagnosis of schizophrenia, schizophreniform disorder, or schizoaffective disorder met criteria for ASD[64]. This is most likely an exaggerated overlap, but it indicates a clinical reality with similar presentations of symptoms. There is at present no larger study of comorbidity of ASD and schizophrenia. A lot of smaller studies of different quality are showing inconsistent overlap[65].

2.2.1 Clinical Picture

ASD are a spectrum of neurodevelopmental disorders characterized by social impairments, communication difficulties, and restricted, repetitive, and stereotyped patterns of behavior[15]. In contrast to schizophrenia ASD becomes apparent in childhood when the child is exposing delayed or deviant language development, lack of interest in social or emotional response to

people, or isn't reaching the expected mile stones of development[66]. Older patients with ASD shows impaired ability to make friends and initiate and sustain conversation. They can exhibit stereotyped, repetitive, or unusual use of language, as well as preoccupation with certain objects or subjects, and inflexible adherence to specific routines or rituals[2]. They have a hard time understanding social cues, interpreting and communicating through body language and facial expressions[67].

2.2.2 Etiology

Genetically there is some overlap between ASD and schizophrenia as well as intellectual disability with common genetic loci and loss-of-function[21, 68-70]. Paternal age, migration, obstetric complications are all associated with both ASD and schizophrenia[71-74]. Both ASD and schizophrenia are more common in males than in females. Family history of schizophrenia is a risk factor for ASD[75-77]. ASD has in common with schizophrenia been shown to be a considerably heritable disorder[24, 78-84]. Advancing maternal age is a risk factor for ASD but not schizophrenia[85].

2.3 PATERNAL AGE

There is convincing evidence that there is an association between advancing paternal age and increased risk of schizophrenia and autism in offspring[86-90]. Other conditions that have been associated with advancing paternal age, but with less robust evidence of an association are birth defects, impaired cognitive and social functioning, schizoaffective disorder, bipolar disorder, substance use disorders, mental retardation, overall morbidity and suicide as well as indications of violent offending and eating disorder[91-101].

In Sweden and large parts of the world the general trend is that fathers are getting older and older before having their first child[102].

Noteworthy is the u-shaped association between paternal age and schizophrenia recurrently seen in studies[87, 88, 103, 104]. The estimates are most of the time not significant, but when pooled together in a meta-analysis the effect becomes significant comparing fathers <25 years to fathers 25-30 years of age (OR=1.06, 95% CI 1.01–1.11)[88].

The population attributable fraction of paternal age to schizophrenia is estimated to >2.5% if the father is >35 years old in one study[25] and 10% if the father is >30 years old and 5% if the father is <25 years old in another study[88].

Thus we have some knowledge about the risk correlated to advancing paternal age (and young paternal age), but what are the mechanisms? There are different theories explaining the paternal age effect. It has been suggested that the relationship between advancing paternal age and schizophrenia is predominantly mediated by genetic factors such as de novo mutations[105], genetic traits associated with both late fatherhood and psychiatric disorders[106], assortative mating[107], or inherited epigenetic factors[108] which will all be described below.

2.3.1 The Environment of an Older Father

An old father is more likely to have been exposed to toxins with potential effect on his offspring. He is also contributing a different psychosocial environment with increased occurrence of adverse life events, e.g. paternal death and illness[109-111]. Even though the environment is not a major suspect regarding the paternal age effect it has not been ruled out and could potentially play a role.

2.3.2 The de Novo Mutation Hypothesis

The de novo mutation hypothesis is referring to the increased risk of mutations and proliferation of these with increased number of cell divisions in the male germ cells with age[112, 113]. This would explain the more or less constant occurrence of schizophrenia in a population even though individuals with schizophrenia tend to have fewer children without compensation with increased number of children by their siblings[114, 115]. The relatively low concordance rate in dizygotic twins compared to monozygotic twins could also be an indication of a mutation[29, 113]. Fathers to patients with schizophrenia were older in families without familial schizophrenia compared to families with familial schizophrenia in some studies[105, 116], but not all[117]. Some studies reports no support of an increased risk of schizophrenia in younger siblings[118, 119] indicating another mechanism than de novo mutations while another study indicate the expected association in a sibling model if de novo mutations is the cause[92]. Studies on mice have shown decreased social and exploratory behaviors in the offspring to older fathers[120] and communication impairments, increased repetitive behavior and social deficits in offspring and grandchildren to older fathers[121]. DNA repair is deficient in spermatozoa of older rats[122]. DNS methylation abnormalities in the sperm of old fathers have been shown in mice[123] and humans[124]. De novo CNV are more common in offspring to older fathers in mice[125]. The mutational burden in human offspring is predominantly due to paternal age[126], but there seem to be no association between CNV load and paternal age in the general population[127]. Thus there are some data supporting the de novo hypothesis even though there is no evidence of a causal relationship between the genetic findings associated with older paternal age and increased risk of schizophrenia.

2.3.3 Delayed Fatherhood/Social Functioning

Another hypothesis is that becoming a father at older age is correlated to certain personality traits (i.e. a distinguishing feature or a person's character, most likely genetically determined) that also correlate to increased risk of schizophrenia in the offspring. Petersen et al found that the association between advancing paternal age and schizophrenia in offspring was confounded by the age of the father when he had his first child[106]. This indicates that the paternal age effect is due to a trait rather than de novo mutations which are merely associated with advancing age. Known endophenotypic deficits (e.g. prepulse inhibition, antisaccade performance, and identical pairs) for schizophrenia showed no increase in endophenotypic deficit levels in schizophrenia with advancing paternal age or paternal age associated difference between affected and non-affected sibling pairs[128, 129]. This is expected if traits rather than de novo mutations is the mechanism behind the paternal age effect. Older

maternal, but not paternal grandfather age adjusted for paternal age is associated with increased risk of schizophrenia[130]. This could be interpreted as a sign of a trait that increases the risk of schizophrenia in offspring and age of parenthood for males, but not females, e.g. a personality trait that is important for timing of family formation in males, but not in females. A suggested trait that has been associated with parental age[96] and schizophrenia[52] is social cognition. Social cognition deficits are common in patients with schizophrenia as well as their siblings, e.g. abnormal emotional processing in siblings to affected individuals[131], social cognition deficits in first degree relatives[132] and parental social cognition (i.e. metalizing) deficits[133], as well as impaired facial affect recognition in first degree relatives[134]. Social cognition is associated with social functioning[135]. Thus there are some data supporting the trait hypothesis even though there is no evidence of a trait that has been connected with delayed fatherhood and increased risk of schizophrenia in the same population.

2.3.4 Assortative Mating

This hypothesis was proposed by Miller et al because of their finding that older fathers were more likely to have children with mothers that had a genetic liability for schizophrenia[107].

2.3.5 Epigenetic Changes

The epigenetic hypothesis is based on the fact that genes can be active or inactive depending on their methylation status. An older father would then have more time to acquire epigenetic changes in the germ cells possibly increasing the risk of schizophrenia in offspring by passing the methylation status to his offspring. This has been shown in the frontal cortex of mice with older fathers[136]. There is also a possibility that different genes are expressed differently depending on if they are inherited from our fathers or mothers, i.e. imprinting. Perrin et al argues that there is an increased risk of imprinting errors in the paternal X-chromosome with advancing paternal age[137]. This could then result in the relatively stronger association between advancing paternal age and schizophrenia in female offspring compared to male offspring[138]. There is also an increased risk of schizophrenia in offspring if the mother is affected compared to if the father is[27, 32] as well as an association between maternal schizophrenia, advancing paternal age and low birth weight[139]. Another way epigenetics might play a role is through the mother's prenatal nutrition[140] or through prenatal stress[141], increasing the risk of schizophrenia in offspring.

2.4 WHAT IS NEEDED

In conclusion, the association between advanced paternal age and schizophrenia in offspring is robust, but there are still questions regarding this association that needs further attention. The mechanism behind the association is not clear. The environmental effects of an older father have not been examined. The evidence that paternal age at first child rather than paternal age per se explains the association has not been widely accepted and needs replication. A mechanism behind the possible association between paternal age at first child and schizophrenia is not known. It is not clear whether the risk factor paternal age is similar in regard to different disorders such as ASD and schizophrenia.

3 AIMS

The general aim of this thesis is to further understand the association between paternal age and increased risk of schizophrenia in offspring. Is there a public health interest to advice men to have children at a younger age in order to decrease the prevalence of schizophrenia and perhaps other disabling disorders and conditions?

3.1 STUDY I

Is the association between advancing paternal age and schizophrenia in offspring due to the environment provided by an older father during childhood?

3.2 STUDY II

Is the association between advancing paternal age and autism spectrum disorder similar to the association between advancing paternal age and schizophrenia? Is there a difference depending on intellectual disability?

3.3 STUDY III

Is the association between advancing paternal age and schizophrenia explained by factors that accumulate over time or factors that are present independent of time and age?

3.4 STUDY IV

Are there personal characteristics that could explain the association between schizophrenia and delayed fatherhood?

4 MATERIALS AND METHODS

This thesis is based on data from registers.

4.1 REGISTERS

In Sweden all residents have a unique personal identification number[142]. This number is used administratively in everyday life in Sweden to identify individuals when they are in contact with authorities, banks, schools, etc. The personal identification number is used when saving data in public registers. This makes it possible to link registers to each other and create databases with vast amount of information. To make sure that individuals won't be identified in these linkage databases the National Board of Health and Welfare or Statistics Sweden replaces the personal identification number with a running number and creates a key to these running numbers. The key is normally kept for a short period and then destroyed. In case the researcher declares a possible need to add information it is possible to postpone destruction of the key three years. This ensures that no individual will be easily identifiable in the database keeping integrity at a high level.

4.1.1 National Registers

4.1.1.1 The Multi-Generation Register (MGR)

The MGR is a nationwide Swedish register held by Statistics Sweden[143]. Individuals born 1932 or later who have been registered as residing in Sweden at any time since 1961 are part of this register as index persons. The register includes birthdates and country of birth for the index persons and links to their biological and adoptive parents. This makes it possible to identify full and half siblings. The coverage is virtually complete for the index persons who have been residents in Sweden since 1968. It is good, but not as complete, for those who were residents sometime between 1961 and 1967, only. The linkage coverage to biological parents is limited when it comes to index persons that were born outside of Sweden and/or were adopted. The register is updated every year. Index persons born 1955-1984 in Sweden are linked in 99-100% to their mother and in 97-99% to their father. In this thesis data collected from the register in 2002 is used, including approximately 9 million index persons[144]. The MGR is part of the RTP.

4.1.1.2 The Register of Total Population (RTP)

The RTP is a nationwide Swedish register held by Statistics Sweden since 1968. It includes demographic information including name, sex, birthdate, address, citizenship, country of birth, immigration, emigration, and date of death[145]. This data collection started when the Swedish church began to keep local registers of its parish members in 1686 [146]. In 1749 it developed into population statistics. The church was still responsible, but collaborated with the state. In 1991 the responsibility for the local registers moved from the local parishes to the local tax-offices. Today the National Tax Board reports changes in the register to Statistics Sweden every month[145].

4.1.1.3 The Swedish Population and Housing Censuses (HC)

Data to the HC was collected by Statistics Sweden through available registers and questionnaires to the total adult (16+ years old) population every five years 1960-1990[147]. It provides individual and household data such as employment, housing, type of household. Coverage was more than 99% except for the last census (1990) where 97.5% of the population participated[148]. Today similar data is collected yearly through different registers and compiled into the longitudinal integration database for health insurance and labour market studies (LISA).

4.1.1.4 The National Patient Register (NPR)

The NPR is a nationwide Swedish register held by the National Board of Health and Welfare containing data on inpatient care at public hospitals, including dates of admission and discharge, main and contributory diagnoses, type of care, sex, and age[149]. It was started 1964 and from the beginning 6 out of 26 county councils in Sweden participated. Psychiatric inpatient care is virtually complete since 1973 with exception from 1984-1986 when data from 5, 2, and 1 county council are missing, respectively. The register became mandatory 1984 through a decision by the Ministry of Health and Welfare together with the Federation of County Councils. The coverage is complete for both somatic and psychiatric inpatient care from 1987. Outpatient data from both public and private caregivers was added from 2001. The coverage of outpatient data is varying and registrations of psychiatric diagnoses is very low before 2006. In this thesis inpatient data from 1973 to 2006 is used.

4.1.2 The Stockholm Youth Cohort (SYC)

The SYC is a cohort of all children 0-17 years old residing in Stockholm 1 January 2001 to 31 December 2007[150]. It is a longitudinal register based cohort with compiled data on children (N= 589 114) and their first degree relatives through record linkage with 16 Swedish national and regional health and administrative registers, including MGR, NPR, and HC described above as well as the VAL database, the Habilitation Register, the Clinical Database for Child and Adolescent Psychiatry described below. By combining national and regional registers it is possible to get more complete and detailed information including perinatal and social characteristics, somatic and mental disorders, legal drug use, sick-leave, disability pension, education and scholastic achievements, and crime convictions. ASD diagnosis in the SYC has been validated through two different methods with good results[150].

4.1.2.1 The VAL Database

The VAL Database is held by the Stockholm County Council and contains information from more than 10 different databases containing information on use of in- and outpatient health care services in Stockholm County[151].

4.1.2.2 The Habilitation Register

The Habilitation Register is held by the Stockholm County Council and contains information from Stockholm County Habilitation Services including type of disability (intellectual disability, pervasive developmental disorder, mobility, and vision or hearing impairments) and use of services[150].

4.1.2.3 The Clinical Database for Child and Adolescent Psychiatry

The Clinical Database for Child and Adolescent Psychiatry is held by Stockholm County Council. It covers child and adolescent (0-18 years old) psychiatric in- and outpatient care and contains information on diagnosis and ratings of general functioning according to the Children's Global Assessment Scale, and in- and outpatient care[150].

4.1.3 The Conscription Register Cohort of 1969 (CRC)

The CRC consists of all Swedish men that were conscripted for compulsory military service between July 1 1969 and June 30 1970, in total 49 321 men 18-21 years of age. All Swedish men were at the time obligated to attend conscription and later military service except about 2-3% who were exempted due to severe disability or illness. The men in the cohort of 1969 went through a more thorough testing than the usual conscription including medical examination, a structured interview with a psychologist, two non-anonymous self-reported questionnaires, and a test of intellectual ability[152]. The first questionnaire addressed social background, upbringing conditions, friendships, relationships, attitudes, adjustment at school and work. The second questionnaire was about drug, alcohol, and tobacco use. Those who had symptoms or reported any mental disorder were examined by a psychiatrist and diagnosed according to ICD-8 when appropriate[153]. The conscription process is a way to determine placement according to aptitude and there is a lot of data regarding social integration, past behavior, and personal characteristics in the conscript register.

4.2 POPULATIONS

More detailed descriptions of the inclusion and exclusion criteria can be found in paper I-IV.

4.2.1 Study I

In study I adopted children were chosen to explore the association between the environment of advancing (adoptive) paternal age and schizophrenia. A cohort of index persons born 1955-1984 who have been registered as residing in Sweden any time up until 2002 was used. The MGR provided data on date of birth for subjects, their parents, and siblings as well as information about biological and adoptive parents. Information on household and family data such as information on level of urbanicity and paternal unemployment was collected from the HC. Data on emigration and date of death was collected from the RTP.

In order to limit the environmental influence of the biological parents adoptees born abroad were included if they had immigrated to Sweden before 2 years of age while Swedish born adoptees were included if their biological mother was known and the adoptee had not lived

with a biological parent at any 5 year point between 1 and 15 years of age. Excluded were adoptees that did not live in a family household or were adopted by a grandparents or siblings. This way the adoptees were more likely to be growing up in families rather than institutions, and the environmental influence of biological relatives were limited. The final sample of 31,188 adopted children (13,405 born in Sweden and 17,783 born abroad) were followed in the NPR from 1973 to 2006 regarding inpatient care for schizophrenia or any non-affective psychotic disorder (Table 2).

4.2.2 Study II

Children born 1984-2001, and residing in Stockholm 1 January 2001 to 31 December 2007 were chosen from the SYC to study the associations between paternal and maternal age, and ASD with or without intellectual disability. Adopted children and children without data on maternal or paternal age were excluded. In the final sample 417,303 individuals were checked in regard of ASD with or without intellectual disability (Table 2) as of 31 December 2007.

4.2.3 Study III

Individuals with one or two paternal siblings were chosen, from the same cohort of index persons born 1955-1984 used in study I, to study the association between advancing paternal age, delayed fatherhood, and schizophrenia. The restriction in number of siblings was to prevent confounding due to the increased risk of schizophrenia in larger families. The study subjects had information regarding both their biological father and mother. Individuals diagnosed with non-affective psychotic disorder or schizophrenia, emigration or death before 15 years of age were excluded due to lack of time-at-risk. First born children and their twins (defined as having the same father and being born the same day or the day after) were excluded to account for the fact that the age at fatherhood (birth of first child) would be the same as the paternal age per se in this group. In the final sample 1,294,063 individuals were followed in the NPR from 1973 to 2006 regarding inpatient care for schizophrenia or any non-affective psychotic disorder (Table 2).

4.2.4 Study IV

Swedish men who went through conscription 1969-1970 and became fathers before 2004 were chosen from the CRC to study the association between personality characteristics and age when becoming a father. Personality characteristics were derived from the self-reported questionnaires and are previously shown to be associated with schizophrenia (i.e. poor social integration and disturbed behavior). Individuals who were diagnosed with psychosis during conscription or lacking information on either poor social integration or disturbed behavior were excluded as well as individuals lacking information on the possible confounders: drug use, IQ, paternal social group, or the family economy. In the final sample 32,306 individuals were followed regarding age at fatherhood until 2003.

4.3 OUTCOMES AND EXPOSURES

Table 1

Exposures and Outcomes in the studies included in this thesis.

Study	Exposure	Outcome
I	Adoptive Paternal Age	Schizophrenia and Non-Affective Psychotic Disorder
II	Paternal Age and Maternal Age	ASD with and without Intellectual Disability
III	Paternal Age and Age at Fatherhood	Schizophrenia and Non-Affective Psychotic Disorder
IV	Disturbed Behavior and Poor Social Adjustment	Age at Fatherhood

4.3.1 Psychiatric Diagnosis

Psychiatric disorders in the NPR are classified in accordance with the World Health Organization's (WHO): International Statistical Classification of Diseases and Related Health Problems (ICD)[154]. It is likely that the criteria from the American Psychiatric Association: the Diagnostic and Statistical Manual of Mental Disorders (DSM)[15] is prevalent in the classification since it has a high impact on psychiatric diagnostic practices in Sweden. However DSM-IV and ICD-10 are similar regarding diagnostic criteria. The diagnosis of schizophrenia and non-affective psychotic disorder was obtained from the NPR and diagnostic data 1973-2006 was used. Diagnosis of ASD and intellectual disability was obtained as of December 31 2007 from four different registers (the NPR, the Habilitation Register, the Clinical Database for Child and Adolescent Psychiatry, and the VAL database) through the SYC. During the follow up period in study I and III there were three different classification systems used, ICD-8[155], ICD-9[156], and ICD-10[154] while ICD-9 and ICD-10 was used during the follow up of study II. The Clinical Database for Child and Adolescent Psychiatry used DSM-IV as classification system until 2008. DSM-IV and ICD-9 use the same codes to classify ASD and Intellectual Disability. The classifications used are presented in table 2. Since a formal ASD diagnosis is a prerequisite before referral to the Habilitation Centers, registration as a service recipient in one of these centers was used as a proxy for ASD diagnosis.

Table 2

Diagnostic Classification used when obtaining information on Schizophrenia, Non-Affective Psychotic Disorder, ASD, and Intellectual Disability from the National Patient Register.

Diagnosis	ICD-8 (1969-1986)	ICD-9 (1987-1996),	ICD-10 (1997-)
Schizophrenia (study I and III)	295 [excluding 295.40, 295.50, 295.70]	295 [excluding 295E, 295F, 295H]	F20
Non-affective Psychotic Disorder (study I and III)	295, 297, 298.20-298.99, 299.99	295, 297, 298C-X	F20-F29
ASD* (study II)		299	F84
Intellectual Disability# (study II)		317-319	F70-79

*Classification according to DSM-IV (299) until 2008 in the Clinical Database for Child and Adolescent Psychiatry

#*Classification according to DSM-IV (317–319) until 2008 in the Clinical Database for Child and Adolescent Psychiatry

4.3.2 Paternal and Maternal Age

Paternal and maternal ages (including adoptive paternal and maternal ages) were defined as age of the parent at the birth of the child. Age at fatherhood was defined as paternal age when the first child was born. Paternal and maternal ages were derived from the MGR, categorized into age groups.

4.3.3 Personal Characteristics

The personal characteristics studied as independent variables in study IV are derived from self-reported questionnaires administered to the CRC 1969. The characteristics were studied regarding association with increased risk of later development of schizophrenia or non-affective psychotic disorder by Malmberg et al[153]. Guided by factor analysis these characteristics were then clustered into two groups with increased risk of non-affective psychotic disorder (including schizophrenia): poor social adjustment and disturbed behavior[152]. These two groups were used in study IV as measures of personal characteristics.

4.3.3.1 Social Adjustment

Social adjustment was based on question regarding current relations and feelings as well as past experiences, including number of friends, being more sensitive than others, and duration of relationship with girlfriend.

4.3.3.2 Disturbed Behavior

Disturbed behavior was based on questions regarding previous misconduct and disciplinary contacts, including running away from home, contact with police or social services, truancy, and lower grades in conduct or discipline at school. Drug abuse was not included in this cluster of questions.

4.3.4 Possible Confounders

Other factors that may influence the risk of developing schizophrenia and possibly be associated with paternal age include demographic information (e.g. place of birth and residence, and socioeconomic status), heritability (e.g. psychiatric disorders in parents and/or siblings), and personal characteristics or behavior (e.g. drug use and IQ). There is a known difference in the risk of schizophrenia related to the sex of the individual. It is important to consider birth year since the time of follow up could differ and there are changes regarding diagnosis as well as inpatient and outpatient treatment over time.

4.4 STATISTICAL ANALYSIS

4.4.1 Logistic Regression

Logistic regression was used to estimate Odds Ratios (ORs) with 95% Confidence Intervals (CIs) in the analysis of the association between advancing adoptive paternal age and adoptee risk of schizophrenia in study I and in the analysis of possible differences between those included in study III and those excluded due to missing data. Logistic regression was used since the outcomes were dichotomous.

4.4.2 Multinomial Logistic Regression

Multinomial logistic regression was used in study IV to estimate ORs with 95% CI for the association between the outcome age at fatherhood, categorized into three groups (<20, 20-35, and 35+ years old), and the exposure of personality characteristics as scores on composite measures of disturbed behavior and poor social adjustment. Multinomial logistic regression was used since the outcome consisted of three groups.

4.4.3 Cox Proportional Hazards Regression

In study III Cox proportional hazards regression was used to estimate Hazard Ratios (HRs) with 95% CI in the analysis of the association between advancing paternal age as well as delayed fatherhood and risk of non-affective psychotic disorder and schizophrenia. Time at risk was defined as time from age of 15 years old to any diagnosis of non-affective psychotic disorder, death, emigration or the end of follow up (year 2006), which ever came first.

4.4.4 Generalized Additive Model (GAM)

In study II penalized cubic regression smoothing splines in a GAM was used to model the associations between the continuous independent variables paternal and maternal age, and the dichotomous dependent variable ASD. Cubic splines were used due to the assumption that the associations were not linear and penalized modeling was used to avoid overfitting the data.

4.4.5 Power Calculation

In study I power calculation was used to determine the chance that the study population was large enough to detect a relative risk of 1.5 with 95% confidence for non-affective psychoses with advanced adoptive paternal age. Power calculation was used since there was no significant difference in outcome between the different adoptive paternal ages (exposure).

4.4.6 Chi-square

In study IV chi-square was used to analyze associations between outcome and possible confounders as well as between exposures and confounders. Chi-square was used since there were multiple confounders and exposures.

4.5 ETHICAL CONSIDERATIONS

Ethical approval of record-linkage data in the cohorts without individual consent was provided by The Research Ethics Committee at Karolinska Institutet, Stockholm, in accordance with the Public Access to Information and Secrecy Act and the Personal Data Act. The former govern when data may be released and the latter how data are used. According to the regulations individual consent is not needed when subjects are not participating actively, the information is treated with secrecy, and the results are presented at group level where no individual is possible to identify. All four studies are register based studies were individuals have been de-identified prior to the researchers access of the data. The results are presented at group level and no individual is possible to identify in the resulting publications. Thus the risk of individual loss of integrity is very small.

In register based epidemiologic studies associations between different factors are studied. It is very important to understand that association does not equal causation and that in communication of study results to the public (including health professionals) this could easily be confused.

5 RESULTS

5.1 STUDY I

Study I found no association between advancing adoptive paternal age and schizophrenia (n=131) or non-affective psychotic disorder (n=371) in adopted children (N=31,188). The only borderline significant result was a lowered risk of non-affective psychotic disorder in adoptees whose adoptive fathers were aged 35–39 years at birth of the child compared to those whose fathers were 30–34 years (OR=0.7, 95% CI 0.6–1.0). The results remained after adjusting for gender, place of birth, and socioeconomic group. Adoptive maternal age and adoptive maternal or paternal psychiatric disorder did not affect the results. The basic assumption that there was no association between age of the biological father and the adoptive father was not contradicted (χ^2=13.5, df=12, p=0.33) in a sample of 24% of the population (7,588 Swedish-born adoptees) for whom there was information on biological paternal age. As expected, in this group the median age was younger for biological than adoptive fathers (26.0 and 35.2 years respectively). There was an increased risk of non-affective psychotic disorder related to advancing biological paternal age, but not advancing adoptive paternal age.

5.2 STUDY II

Study II found that both maternal and paternal age was associated with ASD (n=4746) in a selected sample of the Stockholm Youth Cohort (N=417,303). Advancing maternal and paternal age was associated with greater risk of ASD with intellectual disability (n=1994) than ASD without intellectual disability (n=2752). The maternal age effect was non-linear with increased risk after age 30 (OR=1.07, 95% CI 1.04–1.11 for mothers aged 30–34 and OR=1.75, 95% CI 1.63–1.89 for ages 40–45 compared with the 29-year-old reference group). The paternal age effect was linear (OR=0.93, 95% CI 0.90–0.96 for 25-28 year old and OR=1.14, 95% CI 1.10–1.18 for 40-44 year old fathers compared to 32 year old fathers). For any given age; maternal age was associated with a higher risk of ASD than was paternal age. Maternal age also increased the risk of ASD regardless of paternal age. Paternal age increased the risk of ASD in offspring if the mothers were <35 years old. The model was adjusted for maternal and paternal age, birth year, offspring sex, parity, maternal and paternal psychiatric history, occupational class, family income, maternal region of birth and random effects for biological mother (to account for sibling clustering).

5.3 STUDY III

Study III found that the paternal age effect with increased risk of schizophrenia (n=3,447) or non-affective psychotic disorder (n=8,695) in offspring with advancing paternal age is explained by delayed fatherhood rather than paternal age per se in a selected sample of the Swedish population (N=1,175,941). Advancing paternal age was associated with increased risk of both schizophrenia and non-affective psychotic disorder, but the association was confounded by age at time of becoming a father. Delayed fatherhood as well as early

fatherhood was associated with increased risk of schizophrenia and non-affective psychotic disorder. Compared to the reference group of 25-29 year old first time fathers 40-44 year old first time fathers had an increased risk of offspring with schizophrenia (HR=1.86, 95% CI 1.42-2.45) and with non-affective psychotic disorder (HR=1.60, 95% CI 1.34-1.92), as well as <20 year old first time fathers, who had an increased risk of offspring with schizophrenia (HR=0.71, 95% CI 0.57-0.88) and with non-affective psychotic disorder (HR=0.77, 95% CI 0.77-0.99) adjusted for sex, birthdate, maternal age, paternal age, non-affective psychotic disorder in mother, urbanicity, and paternal unemployment. Non-affective psychotic disorder in father or sibling was added to the analysis as a crude marker of a possible genetic trait. This did not alter the results.

5.4 STUDY IV

Study IV found an association between personal characteristics of poor social adjustment and delayed fatherhood and between disturbed behavior and early fatherhood. There was an association between increasing score of poor social adjustment and delayed fatherhood (OR=2.39, 95% CI 1.96-2.92 for the highest score compared to the lowest). Higher scores on disturbed behavior was associated with becoming a father before 20 years of age (OR=3.78, 95% CI 2.98-4.79 comparing the highest and the lowest score).

6 DISCUSSION

6.1 MAIN FINDINGS IN RELATION TO EARLIER FINDINGS

6.1.1 The Environmental Effect of an Older Father

There was no association between the environmental effect of growing up with an older father and risk of schizophrenia.

6.1.2 Genetics – De Novo Mutations or Traits

The results of this thesis indicate a non-causal association between advanced paternal age per se and schizophrenia in offspring. Our studies indicate that there are alternative explanations behind this association, i.e. one or more factors are likely to increase the risk of schizophrenia as well as influencing age at fatherhood. Study III indicates that paternal age at birth of first child, rather than paternal age per se is explaining the association between paternal age and schizophrenia. This result contradicts the idea that the paternal age effect is due to de novo mutations that accumulate over time. Our results are congruent with a large register-based Danish study[106]. To my knowledge, most studies examining the association have not controlled for age at fatherhood. Thus, there are no studies contradicting age at fatherhood as an explanation for the association between advanced paternal age and schizophrenia.

De novo mutations are more prevalent in older fathers, but up to this point no studies show a direct association between paternal age, de novo mutations, and schizophrenia. The genetic risk burden does not differ between patients with and without family history of schizophrenia[157]. This could be an indication of increased number of de novo mutations in subjects without family history. It could also be viewed as evidence of the heritability of schizophrenia. Indicating that a genetic threshold needs to be reached to develop the disorder and the genetic risk factors of common alleles, i.e. single nucleotide polymorphisms (SNPs), are diffused widely in the population[158]. Studies looking at the genetic risk burden of parents to individuals diagnosed with schizophrenia with and without family history are needed to determine if the risk burden is due to de novo mutations or an unfortunate combination of paternal and maternal genetic risk. Studies looking at paternal age and family history are showing inconsistent results[105, 116, 117].

Social functioning is a trait that hypothetically could explain the association between age at fatherhood and schizophrenia. Social functioning has been associated with both schizophrenia and paternal age in different studies[52, 96]. In this thesis further indication of these associations is presented in study IV. Other indications of social functioning affecting age at parenthood for men more than women are research showing that advancing paternal age is associated with decreased likelihood of male offspring getting married, while the association was less strong for female offspring[159]. Grand paternal age was associated with increased risk of schizophrenia in maternal offspring, but not paternal when controlled for

paternal age[130]. Marriage was also more likely in men with higher income while income did not make a difference in females[159].

6.1.3 Other Findings

6.1.3.1 Assortative Mating

This thesis did not set out to examine if assortative mating is an explanation to the association between advancing paternal age and schizophrenia in offspring. However, in study III the association between age at fatherhood and schizophrenia was not affected when controlled for maternal inpatient treatment for non-affective psychotic disorder. Thus, no support of the theory was found.

6.1.3.2 ASD

Study II indicates that there is a linear association between advancing paternal age and ASD. This association is seen if the mothers are <35 years old, but not in older mothers. There is a greater magnitude of the association between advancing paternal age and ASD with intellectual disability than it is with ASD without intellectual disability. In contrast to what is seen in schizophrenia, maternal age influences the risk of ASD. Previous studies regarding paternal and maternal age, and ASD are inconsistent especially regarding the association between ASD and maternal age[76, 86, 160-165]. Paternal age seems to be of greater magnitude in first born children[166], indicating that delayed fatherhood could play a role in ASD. Studies examining birth order and risk of ASD show inconsistent results[92, 165]. Another difference is that both paternal and maternal advancing grandfather age is associated with increasing the risk of ASD[167]. This holds after adjusting for paternal age, indicating a different mechanism in regard to paternal age than suspected in schizophrenia. Maybe an explanation can be found in regard to the subtypes of ASD. Paternal age was associated with autism while maternal age was associated with Asperger syndrome and pervasive developmental disorder in a study that looked at the subtypes of ASD[168].

6.2 METHODOLOGICAL CONSIDERATIONS

6.2.1 Importance of Replication and Different Study Designs

There is no "perfect" study that is undisputable and will give us all the answers. This might be very clear to you as a reader, but needs to be said. As a consequence there are limitations and uncertainties in all research. When addressing a research question it becomes important to look at it from different angels and to look for information that rejects the hypothesis rather than for information that supports previous beliefs. This is one of the most important differences between scientific reviews and journalistic chronicles. In regard to this thesis it is important that the included studies by themselves are not enough to make any definite conclusions. The studies in this thesis become important in conjunction with other studies. Studies that replicate the findings, studies that use different methods, and studies that view the questions from different angles.

6.2.2 Causality and Confounding

In Sweden we have a lot of public registers that are well suited for research. They include the whole population and cover a lot of different areas. The problem with observational data is that factors that are not studied could be affecting both exposure and outcome, i.e. confound the association. An association in observational data is telling us that there is correlation, but not if there is causation.

A good way to study causal associations is through randomized controlled trails. A group of individuals is randomly chosen to different interventions (exposures). This will theoretically distribute all factors evenly between the groups, except the intervention, especially if the population studied is large. Thus an association between the intervention and the effect (outcome) would indicate a causal relationship. When trying to understand if advancing paternal age is the cause of increased risk of schizophrenia in offspring a randomized controlled trail would include randomizing a group of individuals and controlling at what age they should become parents. This is neither ethical nor feasible in a human population. Since experimental data in humans are not available we have to turn to observational data.

6.2.3 Environmental Confounding, External Validity, and Selection Bias

There is an association between advancing paternal age and schizophrenia in offspring. To find possible mechanisms for this we hypothesized possible explanations. First, the association could be either environmental or genetic. By studying adopted children and the age of the adopting father (study I) we could see if the environment contributed by an older father was increasing the risk of schizophrenia in the adoptee. We found no indication of an environmental effect from an older father during upbringing. Even though the results are convincing there are possible objections especially regarding external validity to study I. Besides this issue of generalizability from adoptive families to the rest of the population there could be a selection bias. Adoptive fathers are not a random sample of fathers in the population. In contrast to other fathers they have been investigated by social services regarding fitness for fatherhood. They are on average older than biological fathers. There could also be differences between adopted and biological children. Even though the environment of an older father doesn't seem to explain the advancing paternal age effect there could still be an environmental effect. The environment (e.g. environmental toxins) is more likely to affect the methylation status of the DNA with time. Through this epigenetic effect there could be an environmental effect influencing the offspring.

6.2.4 Disentangle Possible Genetic Mechanisms

Second, a genetic association could be either paternal traits (i.e. a genetically determined characteristic or condition) or genetic changes in the germ cells with age (i.e. mutational or epigenetic changes). If the increased risk of mutations (or epigenetic changes) with advancing paternal age would explain the association with schizophrenia the risk would be increased regardless of how old the father was when he got his first child. If a trait would explain both being an old father and the increased risk of schizophrenia in offspring we

would see an increased risk with delayed fatherhood regardless of how old the father was when the individual child was born. By comparing age at fatherhood at first child and paternal age (study III) in the same population it is possible to disentangle these two alternative explanations. In our study, delayed fatherhood rather than advancing paternal age per se explained the association. By choosing to analyze only the families with two or three children the increased risk of having a sibling with schizophrenia in larger families was limited. Thus it was possible to examine a crude indicator of familial risk of schizophrenia (i.e. a genetic predisposition). It also introduced a possible selection bias since families with two or three children might be different regarding a lot of unmeasured parameters compared to other families. This could potentially affect the generalizability of the study to families with one or more than three children.

6.2.5 Personality Traits

If it is a trait (or a factor) associated with both delayed fatherhood and increased risk of schizophrenia in offspring, what trait could it possibly be? To study traits (or if you will personality) as risk factors, the best possible way is to measure indicators of interest and then follow the subjects longitudinally. The Conscript Register Cohort of 1969 had measures that could be viewed as crude proxies for different personalities. We know from earlier studies that some of these measures are associated with later development of schizophrenia in the individual. We hypothesized that these personality characteristics are indicative of a genetic vulnerability. In study IV, we tested if the personality characteristics associated with increased risk of schizophrenia was also associated with delayed fatherhood. We found an association and thus an indication of personality traits possibly being the factor associated with both schizophrenia and delayed fatherhood (as well as early fatherhood, actually). This might be a sign of genetic traits, but we cannot be sure that traits are the underlying reason for the difference in the proxy measures we looked at. Environmental effects especially in regard to interactions with other people might also explain the findings. In future studies, it would be interesting to connect the personality characteristics with schizophrenia in offspring, but the time of follow up is too short at present time.

6.2.6 Misclassification Bias

In study I, III, and IV inpatient treatment for schizophrenia and non-affective psychotic disorder was used. There is a risk of some misclassification but most people with schizophrenia has at least one episode of inpatient treatment during the time period of the study and there was no inpatient facility that was exempted from reporting diagnoses to the NPR. The NPR has been validated and found reliable for epidemiological studies[169-171].

6.3 CONCLUSIONS AND FUTURE DIRECTIONS

This thesis adds to the understanding of the association between advancing paternal age and schizophrenia. It strongly indicates that the association is not causal but rather the effect of something that is affecting both age at fatherhood and risk of schizophrenia in offspring. It gives a plausible explanation indicating personal characteristics related to social functioning

as a factor affecting both risk of schizophrenia and age at fatherhood. From a public health perspective this is important since it tells us that an intervention aimed at decreasing the incidence of schizophrenia should not be targeting paternal age per se.

To further understand the mechanisms in common between social functioning, paternal age, and schizophrenia a possible approach could be to focus on the NIMH Research Domain Criteria (RDoC)[172]. In particular the systems for social processes are likely to improve the understanding. By examining the systems for social processes and associating indicators of the different levels (i.e. genetic, molecular, cellular, brain circuitry, physiological, and behavioral) to advancing paternal age as well as schizophrenia (and possibly other neurodevelopmental disorders) it would be possible to improve our understanding.

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