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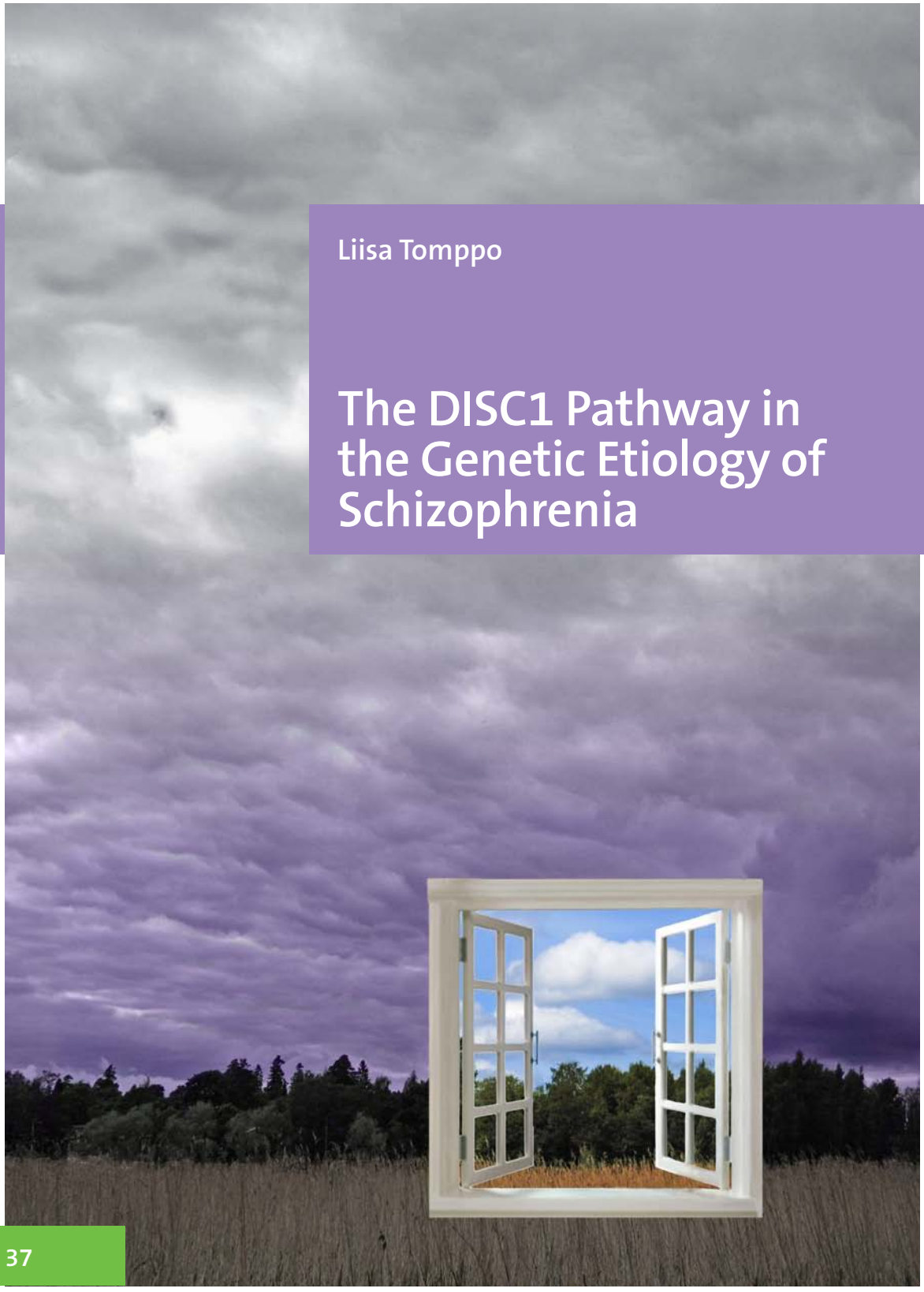

RESEARCH

Liisa Tomppo

The DISC1 Pathway in the Genetic Etiology of Schizophrenia

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The DISC1 Pathway in the Genetic Etiology of Schizophrenia

ACADEMIC DISSERTATION

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To my family

Abstract

Liisa Tomppo. The DISC1 Pathway in the Genetic Etiology of Schizophrenia. National Institute for Health and Welfare (THL), Research 37, 158 pages. Helsinki, Finland 2010. ISBN 978-952-245-315-0 (printed), ISBN 978-952-245-316-7 (pdf)

Schizophrenia is a severe mental disorder affecting 0.5-1% of the population worldwide. The disorder is characterized by positive symptoms, such as hallucinations, delusions and disorganized behavior and speech, and negative symptoms including avolition, anhedonia and flattened affect. Genetic factors have shown involvement in the etiology of schizophrenia. Based on family, twin and adoption studies, hereditability estimates reach 83 %. The genetic etiology of the disorder is complex with evidence for multiple genes and pathways contributing to the onset of the disorder

Many promising candidate genes for schizophrenia have been suggested. In 2000 a novel gene, Disrupted in Schizophrenia 1 (*DISC1*) was recognized at the translocation breakpoint between chromosomes 1 and 11 (t(1;11)(q42.1;q14.3)) in a large Scottish family with the high prevalence of schizophrenia and other psychiatric disorders. Since, *DISC1* has emerged as one of the most promising candidate genes for schizophrenia and other psychiatric disorders based on linkage and association studies and animal models. *DISC1* is also shown to associate with schizophrenia-related cognitive, memory and neuroimaging phenotypes.

DISC1 is a multifunctional hub for many protein interactions involved in intracellular several pathways. A number of genes encoding these proteins have also been identified as promising candidates for roles in the etiology of schizophrenia and other major mental illnesses. This has led to the *DISC1* pathway concept: a hypothesis that proposes that the disruption of the pathways *DISC1* is involved in contributes to risk of mental illnesses and that the disruption of *DISC1* itself is only manner in which DISC1 pathways are affected.

In the present study, we have studied the genes of the DISC1 pathway in the Finnish families ascertained for schizophrenia. We performed a *DISC1* conditioned whole genome-wide linkage study hypothesizing that by conditioning for *DISC1*, we would be able to find new candidates for schizophrenia. We detected significant

linkage on a locus where DISC1 binding partner *NDE1* is located. This finding was followed up and *NDE1* displayed significant association with schizophrenia in the Finnish families.

Encouraged by this finding, we proceeded to study other DISC1 binding partners. Along with *NDE1*, variants in *NDEL1*, *PDE4B* and *PDE4D* displayed significant association with schizophrenia. Additional evidence for these genes has been achieved in many other studies worldwide.

Due to the abundance of evidence supporting the role for *DISC1* in the etiologies of major mental illnesses, we wanted to study the impact of *DISC1* at the population level. We studied intermediate phenotypes for psychosis in the large Northern Finland Birth Cohort 1966. We discovered that in the healthy general population variants in *DISC1* that had previously shown evidence for association with both schizophrenia and bipolar disorder significantly associated with measures of anhedonia, a characteristic negative symptom of schizophrenia.

Since the start of the present study, the number of genes implicated in the DISC1 pathways has grown quickly. Variants in *DISC1* and its binding partners have wide-ranging effects on the expression of numerous genes that they do not even directly interact with. In the last part of this study, we used a conditioned genome-wide approach similar to that used in the discovery of *NDE1* in order to recognize new variants contributing to the etiology of mental illnesses. We hypothesized that with the more homogeneous samples we would be more likely to identify variants that contribute in the etiology of schizophrenia dependent on *DISC1*. We conditioned a genome-wide association analysis on the *DISC1* variants that previously associated with anhedonia. We found evidence for a *DISC1*-dependent association with biological relevance for schizophrenia between physical anhedonia and variants in *CCDC141*, *LCT*, *MIR620* and *MIR128-1*.

The present study supports the involvement of *DISC1* and the DISC1 pathway in the etiology of schizophrenia and psychosis. The study also supports the theory of a complex genetic interplay in the genetic background of schizophrenia with multiple genes in the same pathways contributing both independently and dependently to schizophrenia risk. Based on the association with measures of anhedonia in the general population, the study also suggests that *DISC1* and the DISC1 pathway might have general relevance in human psychological functioning.

Keywords: schizophrenia, psychosis proneness, *DISC1*, DISC1 pathway, association, candidate gene, genetics / psychiatric genetics

Tiivistelmä

Liisa Tomppo. The DISC1 Pathway in the Genetic Etiology of Schizophrenia [DISC1-polku skitsofrenian geneettisenä taustatekijänä]. Terveyden ja hyvinvoinnin laitos (THL), Tutkimus 37, 158 sivua. Helsinki 2010. ISBN 978-952-245-315-0 (painettu), ISBN 978-952-245-316-7 (pdf)

Skitsofrenia on vakava mielenterveyden häiriö, jota sairastaa 0.5-1 % väestöstä maailmanlaajuisesti. Skitsofrenian tyypillisiin oireisiin kuuluvat positiiviset oireet, joita ovat hallusinaatiot eli aistiharhat, harhaluulot, hajanainen puhe ja käytös sekä negatiiviset oireet, joita ovat kiinnostuksen puute, vähentynyt kyky tuntea mielihyvää (anhedonia) sekä tunteiden ilmaisun köyhyys. Skitsofrenian on osoitettu johtuvan osittain geneettisistä tekijöistä. Perhe- ja kaksostutkimusten perusteella skitsofrenian periytyvyyden on arvioitu olevan jopa 83 %. Skitsofrenian geneettinen tausta on osoittautunut monitekijäiseksi. Todennäköisesti skitsofrenian puhkeamiseen vaikuttavat useat geneettiset sekä ympäristötekijät yhdessä.

Useita lupaavia skitsofrenialle altistavia ehdokasgeenejä on tunnistettu. Yksi lupaavimmista ehdokasgeeneistä on *DISC1* (Disrupted in Schizophrenia 1), joka tunnistettiin vuonna 2000, kun kromosomien yksi ja 11 välistä balansoitunutta translokaatiota tutkittiin. Tämä translokaatio, joka katkaisi sittemmin tunnistetun *DISC1* geenin, oli yhteydessä skitsofreniaan ja muihin mielenterveyden häiriöihin skotlantilaisessa suvussa. Sittemmin yhteys skitsofrenian ja *DISC1*-geenin välillä on osoitettu useissa geneettisissä assosiaatio- ja kytkentätutkimuksissa sekä eläinmalleissa ympäri maailman. *DISC1* on yhteydessä myös skitsofreniaan liittyviin kognitiivisiin ja muistiin liittyviin muuttujiin sekä näyttää olevan yhteydessä skitsofreniapotilaille tyypillisiin aivojen radiologisiin muutoksiin.

DISC1 vuorovaikuttaa monien proteiinien kanssa osallistuen useiden aineenvaihduntapolkujen toimintaan. Monet geeneistä, jotka koodaavat DISC1:n kanssa vuorovaikuttavia proteiineja ovat myös osoittautuneet lupaaviksi skitsofrenian ehdokasgeeneiksi *DISC1*:n ohella. Tämä on johtanut DISC1 polku -käsitteen syntymiseen.

Tässä tutkimuksessa kartoitimme DISC1 polun geenejä suomalaisessa perheaineistossa. Ehdollistamalla genominlaajuisen kytkentätutkimuksen *DISC1*:n riskivariantille oletimme, että voisimme tunnistaa uusia skitsofrenian ehdokasgeenejä. Geneettinen lokus, jossa DISC1:n kanssa vuorovaikuttava *NDE1* geeni sijaitsee,

osoitti merkittävää kytkentää. Tarkemmissa analyyseissä *NDE1*:n geenimerkit osoittivat myös merkittävän yhteyden skitsofrenian ja tämän geenin välillä. Tämän löydöksen innoittamina kartoitimme yhteensä 11 muun DISC1:n kanssa vaikuttavan geenin yhteyttä skitsofreniaan. *NDE1*:n lisäksi, *NDEL1*, *PDE4B* ja *PDE4D* geenit osoittivat olevansa yhteydessä skitsofreniaan tässä suomalaisessa perheaineistossa. Sittemmin tukea näiden geenien ja skitsofrenian yhteydelle on löytynyt monissa tutkimuksissa ympäri maailman.

Koska *DISC1*:n ja skitsofrenian väliselle yhteydelle on löytynyt paljon tukea, tahdoimme kartoittaa *DISC1*:n vaikutuksia väestötasolla. Tutkimme psykoosiin liittyviä ilmiasuja laajassa pohjoissuomalaisessa syntymäkohortissa vuodelta 1966. Havaitsimme *DISC1*:n liittyvän kyvyttömyyteen tuntea mielihyvää (anhedonia) myös terveessä väestössä. Anhedonia on myös yksi keskeinen skitsofrenian negatiivisista oireista.

Sen jälkeen, kun tämä tutkimus alkoi, DISC1 polulta tunnistettujen geenien määrä on kasvanut huomattavasti. Lisäksi on havaittu, että *DISC1*:n ja muiden DISC1 polun geenien varianteilla on laajoja vaikutuksia useiden geenien ilmentymiseen, myös sellaisten geenien, jotka eivät suoraan vuorovaikusta DISC1 polun geenien kanssa. Tutkimuksen viimeisessä osassa kartoitimme uusia psykoosialttiuteen liittyviä muuttujia koko genomin laajuudelta. Tälläkin kerralla ehdollistimme tutkimuksen aiemmin tunnistetuille *DISC1* varianteille, kuten teimme tunnistaessamme *NDE1* geenin. Tunnistimme variantteja kolmella geneettisellä alueella, jotka osoittautuivat olevan yhteydessä anhedoniaan ja siten skitsofreniaan sekä assosiaatio- että biologisen tiedon perusteella. Nämä alueet sijaitsivat kromosomeissa kaksi (*LCT, CCDC141* ja *MIR 128-1*) ja 12 (*MIR620*).

Tutkimuksemme tukee *DISC1*:n ja DISC1 polun merkitystä skitsofrenian ja psykoosin taustatekijänä. Sen lisäksi, että *DISC1*:n vaikutus on havaittavissa väestötasolla, onnistuimme tunnistamaan monia muita variantteja DISC1:een liittyvällä polulla. Osa tunnistamistamme varianteista näyttää itsenäisesti liittyvän skitsofreniaan, osa vain ehdollistettuna *DISC1*:lle. Löydöksemme tukevat siten aiempaa käsitystä siitä, että skitsofrenian taustalla vaikuttaa laajamittainen yhteisvaikutus eri varianttien välillä.

Avainsanat: skitsofrenia, skitsofrenian geneettinen tausta / genetiikka, *DISC1*, DISC1 polku, psykiatrinen genetiikka, assosiaatioanalyysi, ehdokasgeeni, psykoosialttius

Contents

Abbreviations

A	Adenosine
AKT1	v-akt murine thymoma viral oncogene homolog 1
ApoER2	low density lipoprotein receptor-related protein 8, apolipoprotein e receptor
ATF4	activating transcription factor 4
ATF5	activating transcription factor 5
cAMP	cyclic adenosine monophosphate
bp	basepair
C	Cytosine
CCDC141	coiled-coil domain containing 141
CEU	Utah residents with ancestry from northern and western Europe
chr	chromosome
CI	confidence interval
cM	centimorgan
CNS	central nervous system
CNV	copy number variation
COMT	catechol-O-methyltransferase
CVLT	California Verbal Learning Test
D’	d-prime measure of linkage disequilibrium
DAOA	D-amino acid oxidase activator
DBZ	DISC1 binding zinc finger protein
DLPFC	Dorsolateral prefrontal cortex
DNA	deoxyribonucleic acid
DISC1	Disrupted in Schizopohrenia 1
DISC2	Disrupted in Schizopohrenia 2
DMD	dystrophin
DTNBP1	dystrobrevin binding protein 1
DRD2	dopamine receptor D2
DSM-III-R	diagnostic and statistical manual of mental disorders - third edition - revised
DSM-IV	diagnostic and statistical manual of mental disorders - fourth edition
ENU	ethyl nitrosurea alkylation
F	frequency
FEZ1	fasciculation and elongation factor 1
FOXP2	forkhead box P2
G	Guanine

GABA	Gamma-amino-butyric acid
GABRA1	gamma-aminobutyric acid (GABA) A receptor, alpha 1
GRIK1	glutamate receptor, ionotropic, kainate 1
GRM3	glutamate receptor, metabotropic 3
GSK3	glycogen synthase kinase 3 beta
HWE	Hardy-Weinberg equilibrium
kb	kilo base
kDA	kilodalton
ICD-8	International Classification of Diseases 8
ICD-10	International Classification of Diseases 10
LC	liability class
LCT	lactase
LD	linkage disequilibrium
MAF	minor allele frequency
MAP1A	microtubule-associated protein 1A
Mb	mega base
miRNA	microRNA
mRNA	messenger RNA
mtDNA	mitochondrial DNA
NDE1	nuclear distribution gene E homolog 1
NDEL1	nuclear distribution gene E homolog like 1
NFBC66	Northen Finland Birth Cohort 1966
NRG1	Neuregulin 1
NUMBL	numb homolog (Drosophila)-like
OR	Odds ratio
P	P-value
PAFAH1B1	platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa
PCNT	pericentrin
PCP4	Purkinje cell protein 4
PCR	polymerase chain reaction
PDE4B	phosphodiesterase 4B, cAMP-specific
PDE4D	phosphodiesterase 4D, cAMP-specific
PER	Perceptual Aberration Scale
QTDT	Quantitative Transmission Disequilibrium Test
r^2	squared correlation coefficient
RANBP9	RAN binding protein 9
RELN	reelin
RGS4	regulator of G-protein signaling 4
RNA	ribonucleic acid
RPAS	Revised Physical Anhedonia Scale

RSAS	Revised Social Anhedonia Scale
SCHD	Golden and Meehl Schizoidia Scale
siRNA	small interfering RNA
snoRNA	small nucleolar RNA
SNP	single nucleotide polymorphism
SzGene	Schizophrenia Gene database
T	Thymine
TRAF3IP1	TNF receptor-associated factor 3 interacting protein 1
tRNA	transfer RNA
TSNAX	translin-associated factor X
TUBA1A	tubulin, alpha 1a
U	Uracile
UTRN	utrophin
WAIS-R	Wechsler Adult Intelligence Scale - Revised
WMS-R	Wechsler Memory Scale – Revised
VTA	Ventral tegmental area
5HT-A1, -A2, 2C	Serotonin receptors A1, A2, 2C

List of original publications

This thesis is based on the following original articles referred to in the text by their Roman numerals:

I Hennah W, Tomppo L, Hiekkalinna T, Palo OM, Kilpinen H, Ekelund J, Tuulio-Henriksson A, Silander K, Partonen T, Paunio T, Terwilliger JD, Lönnqvist J, Peltonen L.: Families with the risk allele of DISC1 reveal a link between schizophrenia and another component of the same molecular pathway, NDE1. Hum Mol Genet. 2007 Mar 1;16(5):453-62.

II Tomppo L, Hennah W, Lahermo P, Loukola A, Tuulio-Henriksson A, Suvisaari J, Partonen T, Ekelund J, Lönnqvist J, Peltonen L.: Association Between Genes of Disrupted in Schizophrenia 1 (DISC1) Interactors and Schizophrenia Supports the Role of the DISC1 Pathway in the Etiology of Major Mental Illnesses. Biol Psychiatry 2009 Jun 15;65(12):1055-62.

III Tomppo L, Hennah W, Miettunen J, Järvelin MR, Veijola J, Ripatti S, Lahermo P, Lichtermann D, Peltonen L, Ekelund J.: Association of variants in DISC1 with psychosis-related traits in a large population cohort. Arch Gen Psychiatry. 2009 Feb;66(2):134-41.

IV Tomppo L, Ekelund J, Lichtermann D, Veijola J, Gabriel S, Järvelin MR, Freimer N, Peltonen L, Hennah W: DISC1 conditioned genome-wide association study on psychosis proneness in a large birth cohort, 2010. *Submitted.*

Publication I has appeared in the academic dissertation by William Hennah.

1 Introduction

Schizophrenia is a severe, debilitating mental disorder affecting approximately 0.5-1 % of the population worldwide. Schizophrenia is a chronic disorder with onset in early adulthood. During its acute phase, schizophrenia causes remarkable reduction in cognitive abilities, psychological functioning and the sense of reality. The disorder causes severe disabilities in one's social and professional lives and ability to care of one self. After the first episode at least 85 % of patients suffer a relapse within 5 years (Robinson et al. 2004).

The etiology of schizophrenia is complex. Evidently several environmental factors contribute to its etiological background together with a large number of genes and genetic factors. The heritability of schizophrenia is estimated to be as high as 83 %, making the genetic component important (Cannon et al. 1998).

Numerous promising candidate genes for schizophrenia have been recognized. One of the most successful findings has been the recognition of the *DISC1*-gene in early 2000. The *DISC1* gene codes a protein that functions as a hub for many intracellular processes. Numerous genes in DISC1-related pathways have been identified and many are good candidates for schizophrenia (Chubb et al. 2008).

The role of the *DISC1* locus has been studied in the Finnish population. *DISC1* displayed significant evidence for association and linkage with schizophrenia in Finnish families ascertained for schizophrenia (Hennah et al. 2003). *DISC1* also associates with schizophrenia-related cognitive phenotypes, such as working memory and reduced gray matter volume, in the Finnish samples (Hennah et al. 2005, Cannon et al. 2005). In addition, evidence suggesting the involvement of *DISC1* in the etiologies bipolar disorder and autism spectrum disorders has been shown within Finnish population (Palo et al. 2007, Kilpinen et al. 2008).

The present study has focused up on studying *DISC1* and the genes involved in the DISC1 pathways in the Finnish population for additional evidence for their involvement in the etiology of schizophrenia.

2 Review of the literature

2.1 Overview of schizophrenia

Schizophrenia is a relatively common psychotic disorder characterized by various symptoms including hallucinations, delusions, apathy, social withdrawal and cognitive deficits. The symptoms result in radically impaired functioning in the affected individuals' social and professional lives and ability to take care of themselves. Patients suffering from the condition are often incapable of work and often require inpatient treatment for extended periods of time (Sadock et al. 2005). Therefore, in addition to schizophrenia being life changing for both patients and their acquaintances, it puts a large burden on mental health services and the finances of healthcare systems (Mueser et al. 2004).

2.1.1 Epidemiology

The prevalence of schizophrenia is quite constant world-wide. In Finland, the life-time prevalence of schizophrenia has shown to be 0.87 % (Perala et al. 2007). The incidence of the disorder in Finland is 0.41-0.53 new cases / 1000 individuals (Suvisaari et al. 1999). The disorder usually develops in early adulthood. The prevalence of the disorder is similar in both sexes, but onset typically occurs later among females. Onset usually takes place ages 20-28 years among males and ages 24-31 years among females (Loranger 1984). Among females the course of the disorder tends to be more benign than in male patients (Mueser et al. 2004).

2.1.2 Clinical features and diagnosis

The clinical manifestations of schizophrenia are diverse. The disorder usually starts to develop with prodromal symptoms. This is a heterogeneous group of symptoms that include: changes in mood; depression; peculiar feelings and thoughts; aggressiveness; sleeping problems; problems in professional and social situations; and inability to concentrate. These symptoms are observed in 75 % of individuals who later develop schizophrenia, and thus they have predictive value for psychosis. However, these symptoms are often moderate and are normal among young adults (Salokangas et al. 2008).

The actual symptoms of schizophrenia can be divided into three dimensions. These include positive symptoms, negative symptoms and cognitive impairment (Mueser et al. 2004)

The typical, psychotic symptoms are referred as positive symptoms, which include hallucinations, delusions and disorganized or catatonic behavior. Hallucinations in schizophrenia are typically auditory hallucinations but visual, olfactory, gustatory

and tactile hallucinations occur as well. Delusions are commonly persecutory delusions, delusions of control, grandiose delusions or somatic delusions. The severity and the duration of positive symptom episodes usually vary (Mueser et al. 2004, Tandon et al. 2009)

Negative symptoms include flattening of speech and affect, avolition (lack of initiative or motivation) and anhedonia (inability to experience pleasure). Negative symptoms are more pervasive than the positive symptoms (Mueser et al. 2004, Tandon et al. 2009, Yung et al. 1996). The predominance of negative symptoms in the acute phase of the disorder has been connected with poorer recovery rates in the future (Thoma et al. 2005).

Cognitive impairment comprises disturbances in memory functions, attention and executive functions. Cognitive abilities often decline rapidly right before and after the onset of the disorder, stabilizing at a level lower than that of the premorbid state. In the best case scenario, with good response to the treatment, cognitive functions might slightly improve in time (Mueser et al. 2004, Tandon et al. 2009).

After the first acute (psychosis) period, approximately 90 % of patients suffer one or more relapses later in life. Cognitive and negative symptoms usually persist for months or years. Thus, schizophrenia is often a chronic disease (Mueser et al. 2004, Tandon et al. 2009).

Schizophrenia diagnoses are based on either the International Classification of Diseases (ICD), currently tenth edition (ICD-10) (World Health Organization 1992) or Diagnostic and Statistical Manual for Mental Disorders (DSM), currently fourth edition (DSM-IV or DSM-IV-TR) (American Psychiatric Association 1994). A new revision of the DSM (DSM-V) is expected to be published in May 2013 (http://www.dsm5.org/Pages/Default.aspx). Classifications by the two systems concur and essentially the two differ only in their semantics (Jager et al. 2004).

According to the DSM-IV criteria (Table 1), the characteristic symptoms of schizophrenia have to be present for at least one month before a diagnosis can be made. Some signs of the disorder, such as prodromal and residual symptoms and disabilities in one's social and professional lives, must be apparent for at least six months.

Table 1. Diagnostic criteria, DSM-IV

A. Characteristic symptoms: Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated):

- delusions
- hallucinations
- disorganized speech (e.g., frequent derailment or incoherence)
- grossly disorganized or catatonic behavior
- negative symptoms, i.e., affective flattening, alogia, or avolition

Only one Criterion A symptom is required if delusions are bizarre or hallucinations consist of a voice keeping up a running commentary on the person's behavior or thoughts, or two or more voices conversing with each other.

B. Social/occupational dysfunction: For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning such as work, interpersonal relations, or self-care are markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic, or occupational achievement).

C. Duration: Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).

D. Schizoaffective and Mood Disorder exclusion: Schizoaffective Disorder and Mood Disorder With Psychotic Features have been ruled out because either (1) no Major Depressive Episode, Manic Episode, or Mixed Episode have occurred concurrently with the active-phase symptoms; or (2) if mood episodes have occurred during active-phase symptoms, their total duration has been brief relative to the duration of the active and residual periods.

E. Substance/general medical condition exclusion: The disturbance is not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.

F. Relationship to a Pervasive Developmental Disorder: If there is a history of Autistic Disorder or another Pervasive Developmental Disorder, the additional diagnosis of Schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month (or less if successfully treated).

2.1.3 Etiology

The etiology of schizophrenia is complex, which means that both genetic and environmental factors play roles in the etiology of schizophrenia. If a close relative is affected with the disorder, the risk of developing schizophrenia is much higher than in the general population. If one's sibling is affected with schizophrenia the risk for developing the disorder is 9 %. Furthermore, if ones monozygotic twin is affected the risk is up to 46 % (Figure 1) (Tsuang 2000). Based on twin, family and adoption studies, the genetic component in the etiology of schizophrenia is strong. In a large Finnish population-based twin study, the heritability was estimated to be 83 % (Cannon et al. 1998). The remaining prevalence is thought to be explained by environmental factors. Currently, it is thought that risk of developing schizophrenia is inherited and the time until onset is dependent on environmental factors (Mueser et al. 2004).

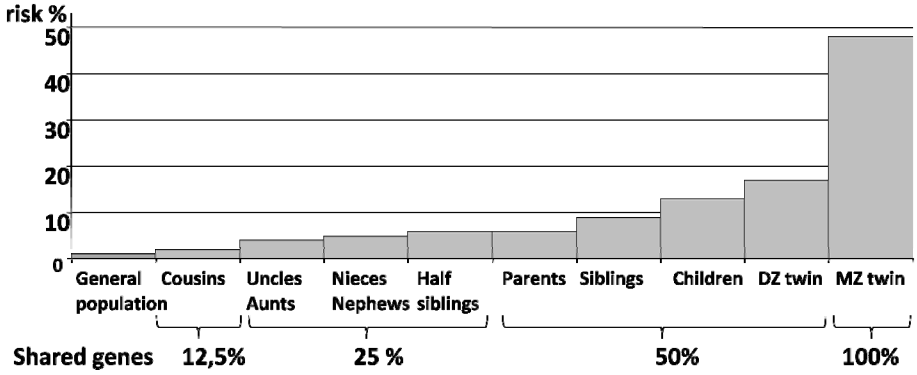


Figure 1. Morbid risks for schizophrenia. Adapted from Tsuang et al. 2000.

2.1.3.1 Neurodevelopmental hypothesis

Schizophrenia is considered a neurodevelopmental disorder in which brain development is disturbed. Schizophrenia patients exhibit minor abnormalities in brain architecture and function. The changes in brain structure are not localized in any specific brain region and are detected at numerous locations. As a result, a concept of schizophrenia as a disease of neuronal connectivity has emerged (Fatemi et al. 2009).

The neuronal migration and neuron development occurring during the embryonic period are disturbed at patients with schizophrenia. The development of the central nervous system is active during childhood when neuronal out-branching and the

formation of new synaptic connections take place. Interneuron deficits are thought to play important roles in the pathophysiology of schizophrenia (Lewis et al. 2005, Lisman et al. 2008, Jaaro-Peled et al. 2009). Postmortem studies of schizophrenia patients' prefrontal cortexes show reductions in GABAergic interneurons (Beasley et al. 1997). The altered maturation of the mesocortical dopaminergic projection is suggested to contribute to the etiology of schizophrenia. Cortical dopamine is suggested to mediate proper information processing and working memory, which are impaired in patients with schizophrenia. Dopaminergic projections from the ventral tegmental area (VTA) to the cortex exhibit marked postnatal maturation (Jaaro-Peled et al. 2009, Rosenberg et al. 1995, Tseng et al. 2007, Egan et al. 2001).

During puberty, a large amount of excess neuronal connections are destroyed (pruning). Subsequently, the reformation capacity of the brain is greatly decreased and minor brain abnormalities might become evident if alternative connections are destroyed. This may explain why schizophrenia often occurs during youth and early adulthood (Fatemi et al. 2009).

Myelination of the cortex occurs postnatally and normally continues until young adulthood, but possibly even longer. Post mortem studies of schizophrenia patients show abnormalities in brain white matter tracts including corpus callosum, anterior commissure and fornix. A decreased number of oligodendrocytes are reported in the cortex and thalamic nucleus, and myelin abnormalities or apoptotic oligodendrocytes in prefrontal regions (Jaaro-Peled et al. 2009, Fields 2008).

Individuals who are affected with schizophrenia have shown in some studies to have neuromotor, language and cognitive developmental deficits already in childhood. These developmental deficits are thought to be genetically programmed, consistent with the concept of schizophrenia as a neurodevelopmental disorder (Jones 1991).

Furthermore, altered brain pathophysiology supports the role of neuronal changes in the etiology of schizophrenia. Brain abnormalities have been reported in the dorsolateral prefrontal cortex, hypothalamus and in different cortical areas. These changes include enlargement of the lateral and third ventricles and reduced gray matter volume, especially in the dorsolateral prefrontal cortex (DLPFC) (Fatemi et al. 2009). Dendritic changes and smaller cell soma have also been observed in patients with schizophrenia (Jaaro-Peled et al. 2009, Glantz et al. 2000, Selemon et al. 1999).

2.1.3.2 Environmental factors

The most studied environmental factors contributing to the onset of schizophrenia are prenatal factors. Stress on the fetus is a risk factor for schizophrenia. Such stress can be caused by infections, the toxic effects of alcohol and drug abuse, and inefficient nutrition among other factors. Complications during the delivery have also been linked to elevated risk of developing schizophrenia. In addition to schizophrenia, these factors predispose to other disorders including mood disorders,

antisocial personality disorder, learning problems and minimal brain dysfunction (syndrome) (Fatemi et al. 2009).

Influenza, retrovirus, rubella and toxplasma gondii infections have been implicated in neuronal development problems and schizophrenia. Viral infections have at least two mechanisms through which they can affect the fetus, and thus affect neuronal development. Some infections are transmitted to and infect the fetus via the placenta, such as infection by the influenza A virus. This can result in altered DNA methylation patterns causing permanent gene expression modifications. The influenza A virus also induces the production of systemic cytokines in the maternal immune system, placenta and fetus, which can result in brain morphology changes (Fatemi et al. 2009).

In addition to prenatal causes, many factors in childhood and youth environments are proposed to contribute to the risk. These include growing up in a dysfunctional family (Wahlberg et al. 1997), an urbanised area or a minority ethnic group (van Os et al. 2009). The use of cannabis and other drugs are suggested to elevate the risk of schizophrenia (van Os et al. 2009). Based on adoption studies, individuals at high genetic risk are more sensitive to problems in the rearing environment than individuals having lower genetic risk (Wahlberg et al. 1997, Tienari et al. 2004). Therefore, environmental stressors are thought to have a particularly deleterious effect on those individuals with a genetic diathesis or predisposition to a particular psychopathology (Tienari et al. 2004).

2.1.3.3 Genetic factors

The search for schizophrenia's genetic component has been ongoing for decades. Despite hard work, no unambiguous variants or risk factors have been recognized. Currently, it is thought that the genetic component in the etiology is complex meaning that multiple genes together with environmental factors contribute to the onset and clinical picture.

Genetic risk factors for schizophrenia will be discussed in more details in Chapter 2.5 Genetics of schizophrenia.

2.1.4 Link between schizophrenia and other mental and neurodevelopmental illnesses

Schizophrenia is one of many in the wide spectrum of psychotic disorders. Other disorders where psychosis is a characteristic symptom are schizophrenia spectrum disorders: schizoid; schizotypal and paranoid personality disorders; schizophreniform; delusional and brief psychotic disorder; and psychosis not otherwise specified. Schizoaffective disorder is a mood disorder with psychotic features. Bipolar disorder and major depression might also include psychotic symptoms.

In families with patients suffering from schizophrenia, other mental illnesses also occur more frequently than in the general population. It has been shown in a Finnish adoptee study that in families ascertained for schizophrenia, the genetic liabilities for both schizoaffective disorder and schizophrenia spectrum disorders are elevated (Tienari et al. 2003). Liability for bipolar affective disorder or major depression was not significantly elevated even though they show some overlap in the genetic risk (Blackwood et al. 2007, Shifman et al. 2004). This suggests that schizophrenia; schizoaffective disorder and schizophrenia spectrum disorders share a common genetic background that predisposes to all these disorders (Tienari et al. 2003, Blackwood et al. 2007, Shifman et al. 2004).

Because of the evidence for the shared genetic factors underlying schizophrenia, bipolar disorder and other psychotic disorders, van Os and Kapur suggested in their recent review the usage of dimensional indicators across diagnostic categories (van Os et al. 2009). They suggested five main dimensions (psychosis, negative symptoms, cognitive symptoms, mania and depression).

Schizophrenia might also share common liability with other disorders. Patients with schizophrenia and autism spectrum disorders have been reported to have similarities in the altered brain development (van Os et al. 2009). Evidence also suggests that part of the genetic liability might be shared with autism since common genes associate with both of these disorders such as *DISC1* and *PDE4B* (Kilpinen et al. 2008, Braun et al. 2007).

2.1.5 Endophenotypes and other alternative phenotypes for schizophrenia

Endophenotypes are intermediate factors between the clinical phenotype and the genotype. These are often used when studying the genetics of complex traits such as schizophrenia, because endophenotypes are presumed to have more straightforward genetic causes than the actual disease phenotype. There are several criteria that an endophenotype has to meet. Endophenotypes must be traits that are associated with the disorder in the population, present in everybody (including the healthy individuals), and are significantly heritable. Endophenotypes must co-segregate with the disorder in families and manifest at higher rate in the family members of affected individuals than in the general population (Gottesman et al. 2003, Braff et al. 2007).

The cognitive deficits schizophrenia patients suffer from provide many phenotypes that can be measured using neuropsychological test batteries. These traits include working memory, verbal memory, attention, information processing, executive functions and sensory gating-related phenotypes (Heinrichs et al. 1998). These cognitive features also appear in milder forms among the healthy relatives of the patients (Hoti et al. 2004) and are heritable (Tuulio-Henriksson et al. 2002, Greenwood et al. 2007). Therefore, they are valid endophenotypes for schizophrenia and also other psychotic disorders (Gur et al. 2007, Antila et al. 2007).

Electrophysiological measures of brain activity are also endophenotypes for schizophrenia. Such measures are event-related potentials P50 and P300, which reflect deflections occurring 50 and 300 ms after auditory stimuli, respectively (Bramon et al. 2004, Patterson et al. 2008). Endophenotypes for schizophrenia also include the cerebral radiological measures: cortical grey matter volume; inter- and intrahemispheric white matter volume; and volume of the brain ventricle (Cannon et al. 2002, Tanskanen et al. 2008).

In addition to the above mentioned verified endophenotypes, other intermediate phenotypes for schizophrenia and psychosis exist. These phenotypes have not been studied extensively enough to meet the strict criteria of endophenotypes.

Many measures have been suggested as indicators of psychosis vulnerability or proneness to psychosis. Using these measures, it might be possible to identify individuals from the healthy population who have a high risk of schizophrenia. Such measures are, for example, the Psychosis Proneness Scales developed by Chapman and colleagues (Chapman et al. 1995). The scales measure properties that are observed at different levels in the healthy population and correspond to positive and negative aspects of psychosis. Patients suffering from schizophrenia score higher than healthy individuals. The scales also vary throughout the clinical state (Horan et al. 2008). They include measures of anhedonia (Physical Anhedonia Scale and Social Anhedonia Scale), which correlate with the negative aspects of schizophrenia. The positive aspects of schizophrenia are measured with the Perceptual Aberration Scale, the Magical Ideation Scale (Horan et al. 2008) and the Schizoidia Scale (Golden et al. 1979).

A number of these scales have their heritability studied. The range of reported heritability estimates for the studied measures are: Revised Physical Anhedonia Scale 0.35-0.57 (MacDonald et al. 2001, Miller 1994), Revised Physical Anhedonia Scale 0.27-0.67 (MacDonald et al. 2001, Miller 1994, Kendler et al. 1991), Perceptual Aberration Scale 0.32-0.40 (MacDonald et al. 2001, Miller 1994). One report concluded familial resemblance of Perceptual Aberration Scale results from shared environmental factors only (Kendler et al. 1991). Many of these scales already fulfill many of the criteria of an endophenotype and further studies may validate them as valid endophenotypes for schizophrenia.

2.1.6 Treatment

The key component of schizophrenia treatment is antipsychotic medication. Antipsychotic drugs can be divided into typical or first-generation antipsychotics (for example haloperidol) and atypical or second-generation antipsychotics (such as risperidone, olanzapine, clozapine and quetiapine). All antipsychotic drugs block dopamine (D2) receptors in the brain and second-generation antipsychotics have

effects on serotonin receptors as well (5HT-2A, 5HT-2C, 5HT-1A). Atypical antipsychotics are currently the most used in the treatment (Salimi et al. 2009)

Antipsychotic medication usually lessens positive symptoms effectively but has less effect on the negative and cognitive symptoms (Salimi et al. 2009, Keefe et al. 2007). Schizophrenia usually requires medical treatment for months or years. Many patients suffer from various side effects of the medication and a large number of them discontinue their medication as a result. Unfortunately many patients do not respond to treatment (Gerretsen et al. 2009).

In addition to medical intervention, different psychosocial therapies are used (Mueser et al. 2004, Kern et al. 2009). Psychosocial intervention seeks to improve the management of schizophrenia and to enhance functioning in areas such as independent living, relationships and work. Specific interventions that have been shown to improve the outcome of schizophrenia include assertive community treatment, family psychoeducation, supported employment, social skills training, teaching illness management skills, cognitive- therapy for psychosis and integrated treatment for comorbid substance abuse (Mueser et al. 2004, Kern et al. 2009).

2.2 The human genome

2.2.1 Overview of the genome

The human genome comprises about 3.2 billion base pairs (bp) divided into 23 chromosome pairs of nuclear DNA and a small 16.5 kilobase (kb) ring of mitochondrial DNA (mtDNA). One half of the nuclear DNA is inherited from the mother and the other half from the father. The mitochondrial DNA is inherited solely from the mother (International Human Genome Sequencing Consortium 2004).

The genetic code of the DNA is determined by the organization of four different nucleobases on a DNA strand; adenine (A), cytosine (C) guanine (G) and thymine (T).

2.2.2 Gene structure and function

A gene has been traditionally defined as a sequence of DNA that codes for a mRNA molecule which is translated into a protein that has an impact on a phenotype (Wain et al. 2002). The human genome is estimated to include 21 000 protein coding genes of an average size of 10-15 kilobase (kb) (International Human Genome Sequencing Consortium 2004, Clamp et al. 2007). However, this definition has proven too narrow, in scope to encompass many newly discovered functional products coded by DNA sequences. According to a recently suggested definition, a gene is a sequence of DNA that codes for a functional molecule, either a protein or a RNA (Gerstein et

al. 2007). The DNA encoding several functional products might have overlapping regions of DNA sequence. A gene is, therefore, a union of DNA sequences encoding a coherent set of potentially overlapping functional products (Gerstein et al. 2007).

The non-coding genes include micro-RNAs (miRNA) and small interfering RNAs (siRNA) (Gerstein et al. 2007). These carry out many regulatory functions and in this way have an impact on gene expression. The miRNAs are small molecules, approximately 20-24 nucleotides long. They are partially complementary to mRNA and can bind to it. By binding to mRNA, miRNAs regulate the expression of their target genes. Usually miRNAs have hundreds of target molecules (Bartel 2004). Like miRNAs, siRNAs are short double stranded molecules which abolish the function of mRNA to which they are homologous (Ghildiyal et al. 2009). Non-coding RNAs also includes tRNA, rRNA (ribosomal RNA), small nuclear RNA (snRNA) and small nucleolar RNA (snoRNA) coding regions (Eddy 2001).

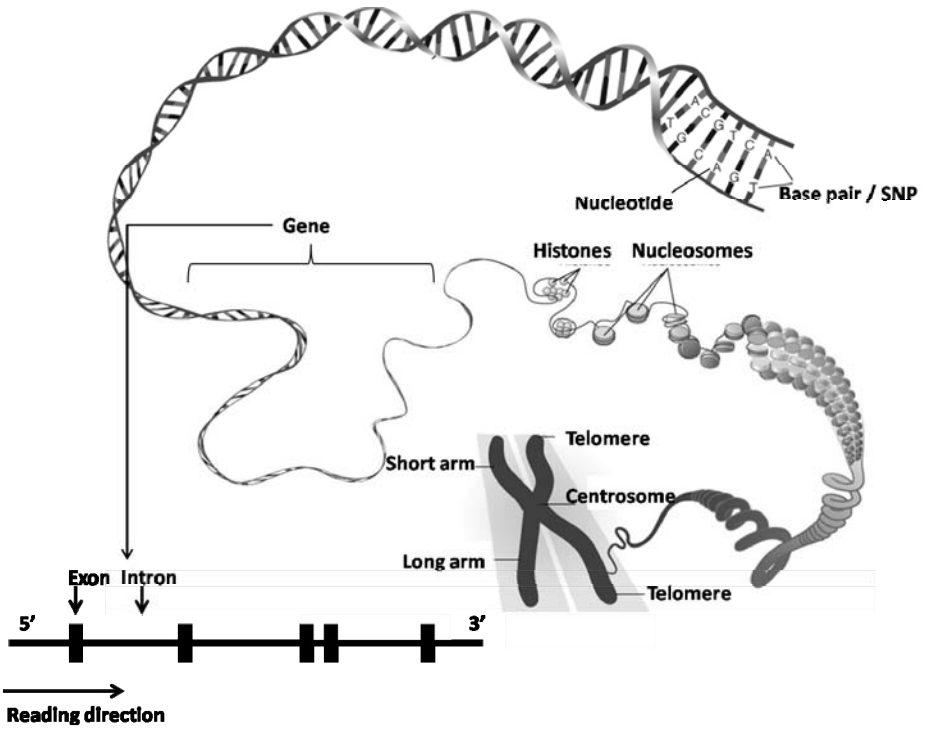


Figure 2. The schematic describes the structure of a DNA strand and its packing into chromosomes. Human DNA is organized into 23 chromosome pairs, which are located in the cell nucleus. A certain sequence of DNA coding a protein is called a gene. The transcription region of a gene comprises of exons and introns. Adapted from National Institutes of Health. National Human Genome Research Institute. "Talking Glossary of Genetic Terms." http://www.genome.gov/glossary/.

2.2.3 Variation within the human genome

Approximately 99.9 % of the human genome is shared among all individuals (Przeworski et al. 2000, Reich et al. 2002). The small portion not shared is responsible for all the variation existing among individuals.

Several types of variation exist within the genome. Variation occurring in the non-coding regions may have no effect on phenotypes. Variation in coding regions, on the other hand, can cause phenotypic differences.

Variation arises in two settings: in the recombination of DNA during meiosis in germ cells, or as a result of structural or sequence mutations occurring at any time point in any cell. Recombination is the main driver of variation transferred to offspring. The further away two genetic loci are on a chromosome, the more likely a recombination even occurs between the two loci. The distance along a chromosome in which a recombination is statistically likely to occur is called Morgan. However, in genetic analyses the smaller subunit centiMorgan (cM) is used, which corresponds to a 1 % chance of change by recombination (Barzel et al. 2008).

2.2.3.1 Structural variation

Structural variation is often defined as genomic variation involving DNA segments larger than one kb (Feuk et al. 2006). The types of structural variation are deletions, inversions, duplications and translocations. Structural mutations can be due to the disrepair of chromosome breaks and the malfunction of the recombination system.

Recombination occurs during meiosis when the double helix of DNA breaks in one maternal and one paternal chromatid, and the ends are switched between maternal and paternal chromatids. Recombination may result in abnormal recombinations of DNA. Recombination might also occur between chromatids of different chromosomes. If both recombinant chromatids end up in the same daughter cell during meiosis, the amount of chromosomal material remains the same in the cell. This type of translocation is called a balanced translocation. An unbalanced translocation occurs when chromatids with translocated material are directed into different daughter cells. In this situation, the chromosome material is either inflated or reduced with respect to the normal amount. Balanced translocations usually do not result in aberrant phenotypes. However, a few exceptions are important. If a translocation occurs within a gene or in close proximity of one, a disruption in the function of this gene might occur (Coop et al. 2007).

Insertions, duplications and deletions over one kb in size differing in copy numbers with respect to the reference sequence are called copy number variations (CNV). This variation often occurs in the repetitive intronic elements of genes. If a CNV is present at a frequency > 1 % in a population, it is called a copy number polymorphism (Stankiewicz et al. 2010).

2.2.3.2 Sequence variation

In addition to structural variation, a large amount of small scale variation exists within the genome. These can be divided into mutations and polymorphisms. In general, mutation refers to variation that alters a phenotype. Polymorphisms are thought to be functionally neutral (Frazer et al. 2009), but the nomenclature is not formally standardized. Small scale variation consists of base substitutions, insertions and deletions. Base substitution is usually found at a single base, but also as a cluster of nucleotides. In deletions, one or more nucleotides are missing from a sequence. Insertions appear when one or more extra nucleotides are found in a sequence. Small scale variation in the population tends to be inherited, but in some cases they can result from new mutations (de novo mutations) (Strachan et al. 2004).

Microsatellites are short tandem repeats of DNA. Dinucleotide repeats are the most common in the genome, for example (CA/GT)*n. The lengths of these repeats often vary between individuals.

Allelic sequence variation that occurs in a significant part of the population (usually > 1%) is called a polymorphism. The most common type of polymorphisms is single nucleotide polymorphisms (SNPs), which are single nucleotides that frequently differ between individuals. Insertions and deletions of one nucleotide are also considered SNPs (Strachan et al. 2004). Over 14 000 000 polymorphic nucleotides have currently been validated (NCBI dbSNP build 131, validated SNPs, http://www.ncbi.nlm.nih.gov/projects/SNP).

Whether SNPs or other mutations have an impact on the phenotype is dependent on the site of the mutation or the polymorphism. In a protein coding region, a mutation might cause a codon change. If the codon change also causes an amino acid change, the mutation is denoted as a nonsynonymous mutation. Nonsense mutations occur when an amino acid coding codon is replaced by a stop codon. If the mutation in a codon causes no change in the amino acid, the mutation is called a synonymous or silent mutation (Strachan et al. 2004).

Mutations in non coding regions have a potential impact on protein coding. Most notably, deletions and insertions can cause a shift in a translational reading frame resulting in a premature termination codon or loss of gene expression (Strachan et al. 2004).

The International HapMap project (Consortium 2003) has collected information on the human genome's common variation by genotyping individuals from all over the world. The HapMap database gathers information on common variation, gene loci, haplotypes and tagging SNPs and publishes it in an easy open-access format. The goal of the HapMap project is to provide a tool for studying common complex disorders, which have been thought to arise from common genetic variation.

It is has recently been proven that a large portion of the genetic liability of common disorders is caused by rare variation. To enable better mapping of rare variants, the 1000 Genomes project was initiated in 2008. In addition to SNP variation, the 1000 Genomes Project will include information on rare and structural variation as well with frequency as low as 1 % in the population. The 1000 Genomes Project is expected to facilitate recognition of functional variants due to its better coverage of the human genome (http://www.1000genomes.org/docs/1000Genomes-NewsRelease.pdf).

Table 2. Examples on the sequence variation within the human genome.

Variation	Description	Example
Single nucleotide polymorphism (SNP)	A single nucleotide in a DNA sequence is replaced with another nucleotide	CGGA**T**GCTGAC CGGA**C**GCTGAC
Duplication	Duplication of a sequence within a DNA sequence	CGGA**T**GCTGAC CGGA**TT**GCTGAC
Insertion, deletion	Insertion or deletion of one or more nucleotides from a DNA sequence	CGGA**T_**GCTGAC CGGA**TC**GCTGAC
Microsatellite/ minisatellite	Tandem arrays of repeat units	CGGAT**GC**TGAC CGGAT**GCGCGC**TGAC

2.2.3.3 Epigenetic variation

For decades it was believed that variation within the DNA sequence is the only type of variation that is heritable. However, new studies have shown that different kinds of DNA modifications can be inherited, which alter phenotypes in following generations by affecting gene expression. In addition to the genome inherited from parents, an individual inherits an epigenome. DNA methylation is one of the best studied epigenetic mechanisms. Methylation makes chromatid closed, transcriptionally inactive sites. CpG islands are methylation important targets in humans. DNA methylation is an important factor in the etiology of Rett's syndrome, mental retardation and fragile X syndrome (Bird 2002, Klose et al. 2006). Along with DNA methylation, histone modifications are important epigenetic mechanisms. They include various kinds of covalent modifications such as lysine acetylation, methylation, SUMOylation, and ubiquitinylation; arginine methylation; serine

phosphorylation; and proline isomerization. The different covalent modifications have different effects on gene expression for instance, the activation of gene expression by acetylation and the repression of gene expression by SUMOylation (Roth et al. 2009, Turner 2002, Li et al. 2007a).

Variation also appears after transcription. For instance, alternative splicing causes variation by the numerous splicing combinations of a pre-mRNA molecule. Over 80 % of human genes are alternatively spliced and the most common splicing mechanism is exon skipping (Black 2003, Matlin et al. 2005).

2.3 Genetics of complex disorders

In the simplest case, a mutation in a gene always causes the same phenotype. Disorders caused by single genes are called monogenic or Mendelian disorders. They follow a dominant or a recessive model of inheritance. In dominant inheritance, a certain variant in a gene causes a phenotype independent of the other variant present in the gene pair. In recessive inheritance, a phenotype is expressed only if both alleles carry the same variant. Some important exceptions arise due to incomplete penetrance and imprinting. Penetrance is the fraction of the dominant genotypes manifesting in given phenotypes in a population. In some cases, recessive phenotypes display dominant inheritance pattern. This is due to genetic imprinting, a phenomenon where only one of the gene copies is expressed. Either the paternal or the maternal chromosome is silenced, and thus a single mutation in the active gene is sufficient to cause a change in phenotype. Monogenic disorders are usually rare.

The vast majority of common diseases are polygenic or multifactorial (complex) disorders. Many genes or genes together with environmental factors contribute to the onset of a disorder. An individual might carry several predisposing and protective variants in his or her genome and the combination of these variants together with environmental factors results in the actual disease phenotype.

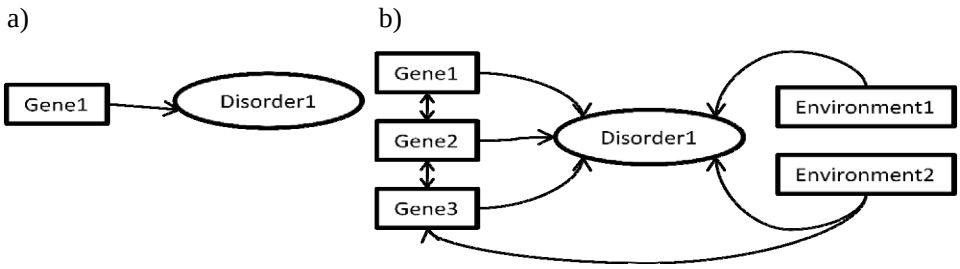


Figure 3. The schematic illustrates concepts of a monogenic (a) and a complex (b) disorder. In monogenic disorders, a variant (or a few different variants) in a single gene causes the disorder phenotype. In complex disorders, both genes and environmental factors contribute to the phenotype together.

In complex disorders, the role of the genetic background varies from disorder to disorder. Heritability (the proportion of the phenotypic variation attributable to genetic variation) of a complex disorder can be estimated based on twin, adoption and family studies. The most common way of estimating the proportion of inheritance is to study monozygotic and dizygotic twin pairs. The heritability h^2 (narrow sense heritability) can be expressed as the difference in concordance between monozygotic and dizygotic twins:

$$h^2 = \{(mz\ concordance) - (dz\ concordance)\} * 2$$

This gives an estimate for the upper limit of heritability.

The variation of a DNA sequence is usually studied by genotyping markers throughout the region of interest and analyzing them using linkage or association methods. The following introduces the main methods and study designs for complex disorders.

2.3.1 Linkage studies

Linkage is a measure of whether the transmission of certain genetic markers occurs together or not. During meiosis, recombination between chromosome pairs might occur. The further away from each other two chromosomal loci are, the more likely it is that recombination occurs. The recombination fraction which is the probability that a gamete produced by a parent is a recombinant is a measure of genetic linkage. For loci that are located on different chromosomes the recombination fraction (θ, theta) is 0.5. If two loci are located just next to each other on a chromosome, it is unlikely that recombination occurs and the recombination fraction is 0. Thus the recombination fraction has values $0 \leq \theta \leq 0.5$.

In genetic studies, it is often impossible to count recombination fractions for each individual in each pedigree. Usually, an overall likelihood for a family is calculated

the alternative assumptions being θ = 0 (two loci are linked) and θ = 0.5 (two loci are not linked). This is the odds of linkage. The logarithmic value of this score gives the logarithm of odds known as the LOD score. In a sample including numerous families, LOD scores are added up together across the families (Strachan et al. 2004).

$$Z(\theta) = log_{10} \frac{L(\theta)}{L(\theta = 0.5)}$$

Usually a LOD score of ≥ 3.3 is considered as the limit for statistical significance and corresponds to the significance level of 0.05 (Lander et al. 1995). When studying a disease phenotype, the co-segregation of a disease with a certain genetic locus is studied in the same manner as when studying two genetic loci. In linkage studies, microsatellite markers are usually studied. They can be used for both genome-wide linkage studies and the denser fine mapping of certain chromosomal regions.

Since linkage refers to the transmission of studied alleles within families, linkage analyses can only be performed within family samples with both offspring and parental genotypes available.

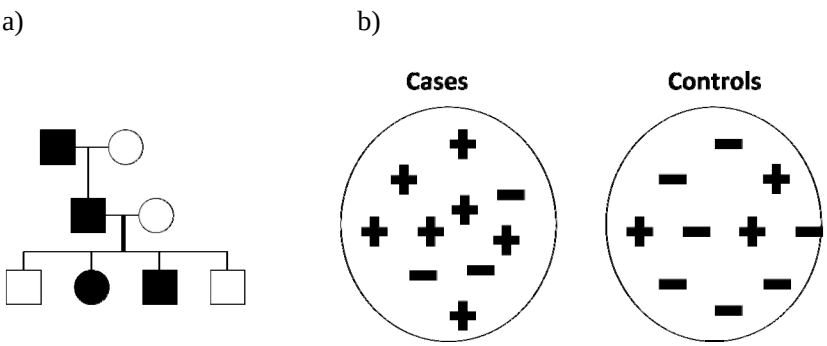


Figure 4. Simplified models of the concepts of linkage (a) and association (b). Linkage means that a certain variant is inherited in a family together with a disease. Therefore, the affected individuals (filled symbols) share a common genetic variant. Association is simply a correlation between two studied measures like a genetic variant and a disorder, for example. However, association can also be detected within families (a) when the same allele of the variant associated with the disorder.

2.3.2 Association studies and linkage disequilibrium

Association analysis is a statistical method that tests if certain alleles occur together with a given disease or phenotype. While linkage analyses can only be performed in families, analyses of association can be performed in all kinds of samples. Both dichotomous and quantitative phenotypes can be studied.

In case control studies, a simple chi squared test can be performed to test for association between genetic markers and the disease phenotype. If the phenotype is a continuous quantitative trait, such as height and weight, regression models can be used to testing for association. The matching of the cases and controls is critical in genetic studies. Poorly matched controls can result in false results (Balding 2006).

In family studies, if at least one parent is genotyped, a transmission disequilibrium test (TDT) can be used. It compares the transmission of alleles from parents to affected offspring. The TDT test can be performed both for dichotomous and quantitative traits.

Usually, the genetic variation studied in association analyses are SNPs. Since the human genome includes over 14 000 000 SNPs according to current estimates (NCBI dbSNP build 131, validated SNPs, http://www.ncbi.nlm.nih.gov/projects/SNP), every 200th nucleotide is polymorphic, and thus testing all the markers within a region of interest would be unreasonable. However, not all SNPs are independent. If no recombination occurs, neighboring SNPs are inherited as a block called a haplotype. The non random association of alleles is called linkage disequilibrium (LD). LD can be observed within chromosomes and between different chromosomes (Mueller 2004).

LD in a population can be observed as block like structures (LD or haplotype blocks) throughout the genome that are flanked by regions of high recombination frequency (International HapMap Consortium et al. 2007). Due to recombination, haplotype blocks become shorter with every generation. A majority of haplotypes within LD blocks are shared within populations and these common haplotypes account for the vast majority of the genome (Jakobsson et al. 2008). By selecting and genotyping a single SNP, information on the other SNPs within the same haplotype block is captured simultaneously. SNPs that describe a haplotype's variation are referred to as tagging SNPs (tag SNP / htSNP).

LD can be measured using several methods. The most frequently used pair-wise measures of LD are the D prime measure of LD (D') and the squared correlation coefficient (r^2). The measures of r^2 and D' are used for different purposes. D' is a useful tool for estimating the probability of historical recombination and for recognizing ancestral variants in a given population. The measure of r^2 is useful in association studies (Balding 2006, Mueller 2004).

The D' measure of LD is advantageous for haplotype block estimation and construction. Many approaches to the estimation of D' based LD block structures

within family data have been suggested, including the D' confidence interval and the solid spine of LD (Gabriel et al. 2002, Barrett et al. 2005). The haplotype structure can also be estimated using a four-gamete test-based application (Wang et al. 2002). In association analyses and linkage disequilibrium tests, haplotypes can be more informative than genotypes of single markers (Balding 2006, Gao et al. 2009, Akey et al. 2001). Since, variation in the population is structured into haplotypes which are inherited as blocks it is reasonable to study these blocks instead of studying single SNPs (Clark 2004). Haplotypes lead to analysis with fewer degrees of freedom but on the other hand this advantage is minimized if only the tagging SNPs are included in the analyses (Balding 2006, Clark 2004). Further, haplotypes are suggested being able to capture the combined effects of tightly linked cis-acting causal variants (Balding 2006, Clark 2004).

Both r^2 and D' measures can be utilized for the selection of tag SNPs. In general SNPs with LD of D' $\geq$ 0.8 or $r^2 \geq 0.8$ are considered to tag each other with sufficiently high accuracy, especially if family (trio) data is available. In small samples, LD might be overestimated by D' and underestimated by r^2 (Mueller 2004, He et al. 2009).

In the best case scenario, an association analysis identifies a functional, phenotype-causing variant. The identified variant would cause an amino acid change in a protein, and thus change the protein's function such that it predispose or outright causes a disease. In complex disease genetics, this is rarely the outcome of association analyses. The main goal in association studies is to discover loci that display association with the studied trait and are in linkage disequilibrium with the actual trait locus genotype (Figure 5). The associated markers thus tag and capture the genetic variation involved in the etiology of a disease or trait (Balding 2006).

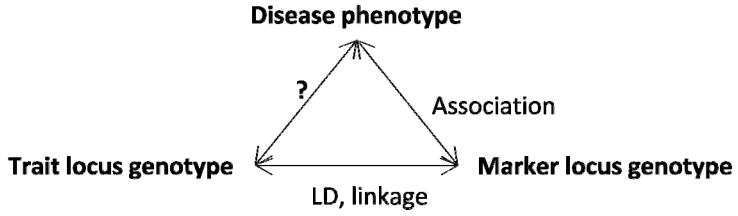


Figure 5. A schematic model of an association study of a complex disorder. In association studies the recognition of actual functional variants is not often possible.The main goal is the identification of loci that correlate with the measured phenotype, which then suggests that the actual causative variant is in LD or is linked with the associating variant. Therefore, the relationship of interest is actually the one between the phenotype and a trait locus genotype (indicated with a question mark).

2.3.2.1 Genome-wide studies

In genome-wide studies, genetic markers through the whole genome are analyzed. These studies are performed using both linkage and association methods. Genome-wide linkage scans were previously widely used and they have helped identify several important disease loci.

Recently, genome-wide association studies have become a central study design and analytical method in human genetics. They enable genotyping and analyzing of a large number of SNPs throughout the genome. Genome-wide SNP assays currently include up to 1 000 000 markers (Illumina, San Diego, CA, US / Affymetrix, Santa Clara, CA, US) .

2.3.2.2 Candidate gene studies

Following the candidate gene approach, specific genes are studied based on the hypothesis of their involvement in the etiology of a given disorder (Tabor et al. 2002). The susceptibility loci have often been recognized via genome-wide linkage or association studies. Another method for selecting candidate genes is selection based on biological evidence or pathway information. If a gene has displayed evidence for association with a disorder, it might be of interest to study the genes involved in the same pathways or those that have similar functions to the identified genes. Similarly, if a protein is hypothesized to be involved in the etiology of a disorder, a gene coding for the protein might be a suitable target for a candidate gene study (Tabor et al. 2002). Genetic markers are genotyped in coding sequences, flanking regions and possibly splice and regulatory sites of candidate genes in order to capture variants driving differences in disease prevalence (Balding 2006).

2.3.2.3 Statistical significance

In association studies, a generally accepted limit for statistical significance is $P < 0.05$ after correction for multiple testing. A P-value below 0.05 leads to the rejection of the null hypothesis and approval of the alternative hypothesis. Current methodology enables the testing of a large number of genetic variants in large samples. Genotyping platforms have allowed for the detection of variants with smaller effect sizes by enabling the testing of even larger samples with more markers. However, the new techniques have led to false positive results and have made multiple testing a reoccurring critical problem. When performing a large number of tests, the likelihood for achieving "a significant result" by change increases. Therefore, if 100 tests are performed five of these are expected to display P-values < 0.05 by chance.

A rejection of the null hypothesis when it is actually true is type I error (or a false positive or α-error) and leads to the false interpretation of a finding as significant. In contrast, type II error (a false negative or β-error) refers to the failure to reject the

null hypothesis when it is not true. Type II thus refers to the failure to detect a significant difference between the disease and its genetic variant even though a significant difference exists.

To avoid type I and II errors, multiple testing must be controlled and corrected for. The Bonferroni correction is considered the most conservative way to correct for multiple testing. The correction takes into account the total number of the tests performed and assumes that all tests are independent. While the Bonferroni correction effectively reduces the rate of false positive, it can result in false negative results by being overly conservative. In genetic analyses, the analyzed markers or the traits are rarely fully independent and thus the assumption of the test is not fulfilled (Balding 2006). Nyholt and colleagues proposed a new method in 2004 for estimating the number of independent markers and traits in analyses. Instead of using the actual number of performed tests in the correction, estimated numbers of independent tests are used (Nyholt 2004).

Empirical P-values can be estimated using a permutation correction. The permutations test the likelihood of observing the achieved P-value by chance by randomly testing for the association between the genotype and the phenotype. The result is dependent on the number of replications performed. Generally, the accuracy of the test increases with the number of permutations, which must at least be the number of the tests performed (Balding 2006).

Replication of the original finding in an independent study material is considered adequate proof of a true positive result. In the best case scenario, replication with the same allele of the same marker and with the exactly same phenotype is performed (Burmeister et al. 2008). Interpretation difficulties often arise with negative replication results, especially when studying complex traits if the markers studied are not exactly the same, the sample sizes are different and the phenotypes are estimated differently. These kinds of findings are often not considered sufficient enough to disprove a previous positive finding. If the size of the original study sample is large enough, replication can already be performed in the original study by splitting the sample into explorative and replication study groups.

Study specific limits for statistical significance can be estimated using simulations based on material characteristics (Wilkinson 2009).

In complex disorders, the effect size of single genetic variant is often small. Not all affected individuals carry a certain risk variant and the risk or susceptibility variant is often also present in the healthy population. The impact of the variant can only be reliably detected in large samples. The power of the study describes the probability of successfully detecting an effect of a particular size (Purcell et al. 2003). If β is the probability of type II error, then power is $1 - \beta$. Power depends on several factors including the magnitude of effect, sample size and required level of statistical significance (α) (Purcell et al. 2003). Power calculations can be performed *a priori* to determine the sample size needed for the study to detect variants of certain effect

sizes (Berry et al. 1998). *Post hoc* power calculations may show whether a study was underpowered, which can be useful in interpreting negative results (Purcell et al. 2003). Post hoc analysis can also be used for determining the smallest detectable effect in a particular study sample (Purcell et al. 2003).

2.3.2.4 Meta-analyses

In meta-analyses, the results of multiple studies with similar hypothesis are combined. Results are reanalyzed by modeling effect sizes dependent on sample size in each study and estimating the overall significance using regression models (Munafo et al. 2004). The advantage is the increased power attained with larger sample size. Meta-analyses also enable the verification of association findings across populations. However, meta-analyses are prone to publication bias by referring to studies with positive results that are more likely to be published than papers with negative results. Furthermore, strong individual signals may overwhelm weaker ones in the combined analyses (Normand 1999). A crucial question for any meta-analysis is also the degree of heterogeneity that exists between individual studies. In meta-analyses, differences in study designs and populations' genetic backgrounds may result in uninformative outcomes (Munafo et al. 2004). In studies of psychiatric disorders the phenotyping method is especially critical, because studies that define phenotypes differently may not be combinable in meta-analyses.

Imputation is widely used in concert with meta-analyses. Imputation is used to estimate the genotypes of SNPs based on the surrounding SNPs' genotypes and HapMap LD information (Servin et al. 2007). Imputation is particularly useful for combining results obtained from different genotyping platforms (Li et al. 2009). Therefore, imputation can be a useful tool in conducting meta-analyses across studies.

2.3.3 Population isolates in complex trait mapping

A population isolate refers to a population that originates from a small number of founders, experiences little immigration and expands primarily through population growth. As a result, the genetic variation within an isolated population is less than in more mixed populations (Norio 2003).

Population isolates provide many advantages in genetic studies. In the case of a monogenic disorder, a single genetic variant might be responsible for all cases in the population (a consequence of the genetic bottleneck effect). Therefore, the recognition of disease variants in such a setting is easier than in more mixed populations. The number of causative variants of complex disorders is suggested to be smaller in isolated populations. Further, the recognition of genetic regions involved in the etiology of a certain complex trait is facilitated by the high level of LD within the population (Kaessmann et al. 2002). However, the recognition of the actual disease causing variants of complex disorders may be challenging since the

genetic markers showing significant association can be far away from the actual disease locus (Kaessmann et al. 2002). It has thus been suggested that populations with high LD are ideal for the coarse mapping of disease loci (Kaessmann et al. 2002). However, the susceptibility variants in an isolated population may differ from those in other populations. Therefore, results from an isolate may not reflect the underlying genetic etiology of the disease in other populations.

Several isolated populations have been studied and utilized in the recognition of genetic variants underlying diseases. These populations include the Finnish population (Peltonen 2000), the Saami (Laan et al. 1997), the Sardinian population (Angius et al. 2001), Old Order Amish (McKusick 1973) and Jewish communities (Bonne-Tamir et al. 1986).

The Finnish population has remained isolated until relatively recently and there are several internal isolates within Finland. Finland was inhabited approximately 10 000 years ago. The major groups making up the current Finnish population are the eastern and western populations, which inhabited Finland 4000 and 2000 years ago, respectively, according archeological findings (Varilo 1999). For a long time, only the coastal regions were inhabited in Finland. As late as the 16th century, the eastern and northern parts of Finland were inhabited. Genetic drift in these small sub-populations along with genetic bottle necks, such as the great famine in the years 1696 - 1698, has greatly affected the Finnish gene pool (Varilo 1999).The Finnish population shows decreased genetic diversity and increased LD compared to other European and especially African populations (Peltonen 2000, Lander et al. 1994). However, some smaller isolated population for example the Saami display even more extensive LD than Finns (Kaessmann et al. 2002). Furthermore, even though the Finnish population is more homogenous than many other populations, fine-scale population substructures can be observed within Finland (Jakkula et al. 2008).

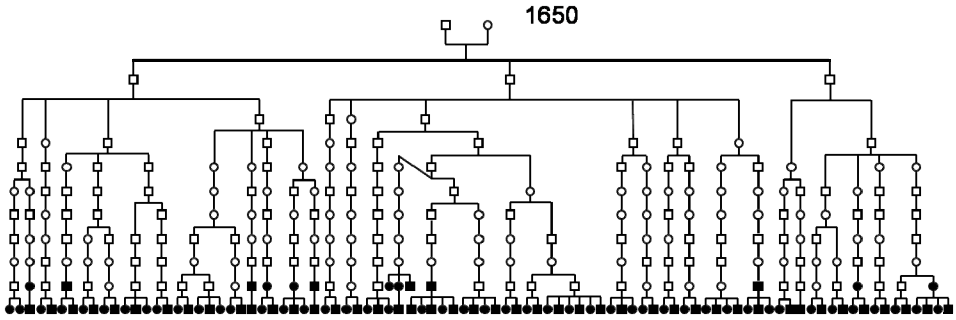


Figure 6. The schematic describes a schizophrenia pedigree from a Finnish isolate. All the affected individuals (filled symbols) share common ancestors dating back to 1650 (Hovatta et al. 1999). Only the relatives connecting individuals in the pedigree are shown. Pedigree figure courtesy of Teppo Varilo.

2.4 Genetics of schizophrenia

The search for the complex genetic component in the background of schizophrenia has been ongoing for decades. To date, no unambiguous variants or risk factors have been identified. However, many promising candidate genes have been recognized in linkage and association studies. All the recognized variants within these genes explain only a small proportion of the genetic risk. Furthermore, all these genes include several variants that have been reported to either increase or decrease the risk of developing schizophrenia. Therefore, there is evidence of significant genetic heterogeneity, which is the phenomenon of a large number of genes and gene variants contributing to a trait and different variants within the genes resulting in similar phenotypes. All these variants and their interactions together may have an impact on the etiology of schizophrenia along with contributing environmental factors.

Many of the candidate genes have been recognized using linkage studies. Schizophrenia linkage studies have pointed to almost all chromosomal regions, which might be evidence of genetic heterogeneity (Sullivan 2005). However, meta-analyses have detected consistent evidence of linkage on some chromosomal regions. Table 3 summarizes results from two genome-wide linkage meta-analyses (Lewis et al. 2003, Ng et al. 2009). Promising candidate genes for schizophrenia from linkage studies include *RGS4* on chromosome 1q23.3, *DAOA* on chromosome 13q33.2, *NRG1* on chromosome 8p12 and *DPTN1* on chromosome 6p22.3 (Schwab et al. 2009).

Association analyses are currently the most commonly used method for studying the genetics of schizophrenia and other disorders with a complex genetic background. The Schizophrenia Gene (SzGene) database summarizes current evidence from schizophrenia association studies. As of February 2010, it has information on 953 candidate genes, 1593 studies and 267 meta-analyses. Table 4 summarizes the top 43 candidate genes and regions listed in the SzGene database as of February 2010 (Allen et al. 2008).

Large scale genome-wide association studies were expected to recognize potential new genetic risk factors and gather more evidence for the previously identified suggestive findings. Instead, all genome-wide studies have displayed only suggestive evidence for association at many different loci. All the recognized variants have displayed only small effect sizes, a result that is in line with previous candidate and linkage studies (Porteous 2008).

Recent studies have suggested that in addition to common variants with small effect sizes, a large component of the genetic etiology of schizophrenia might be explained by rare variants with small effect sizes. Such variants include copy number variation (CNV) and de novo mutations. Rare deletions at chromosomes 1q21.1, 15q13.3, 15q11.2 and 22q11.2 and rare duplications at chromosomes 16p11.2 and 16p13.1

increase the risk for developing schizophrenia(Kirov 2010, McCarthy et al. 2009, International Schizophrenia Consortium 2008, Stefansson et al. 2008, Kirov et al. 2009a, Rujescu et al. 2009, Kirov et al. 2009b, Bassett et al. 2008). These variants are collectively found in 3 % of the patients studied, and thus they only explain a small proportion of the genetic causes of schizophrenia (Kirov 2010). Large rare (>100 kb) CNVs that disrupt genes are signicantly more frequent among schizophrenia patients than among healthy controls, and the genes disrupted tend to affect neurodevelopmental pathways (International Schizophrenia Consortium 2008, Walsh et al. 2008). Furthermore, de novo CNVs are significantly more common in sporadic schizophrenia cases than healthy controls (Need et al. 2009, Xu et al. 2008).

In a few cases of patients with schizophrenia, large scale chromosomal abnormalities have been detected. Schizophrenia candidate genes *DISC1* and *PDE4B* were originally recognized at chromosomal translocation break points. *DISC1* was recognized in the year 2000 on chromosome 1 in a balanced translocation between chromosomes 1 and 11 that co-segregated with mental illnesses in a large Scottish pedigree (Millar et al. 2000). Likewise, *PDE4B* was recognized at a balanced translocation break point between chromosomes 1 and 16 in a Scottish family (Millar et al. 2005).

Table 3. Linkage meta-analyses in schizophrenia. The table summarizes the chromosomal regions displaying nominal evidence for linkage in meta-analyses of partly over-lapping genome-wide studies (Ng et al 2009 and Lewis et al 2003). The regions displaying P-values < 0.05 are displayed. Results that are significant and suggestive at the genome-wide level are indicated. The Study by Ng et al included a total of 32 studies with a sub sample of 22 European studies. The study by Lewis et al included 20 studies.

				Genome-wide sign.		
Study	Bin	Chromosome	cM	Significant	Suggestive	Samples
Ng2009	5.6	5q31.3-35.1	148.9-178.7		+	32
Ng2009	2.5	2p12-q22.1	117.5-146.9		+	32
Ng2009	1.6	1p13.3-q23.3	143.1-171.7			32
Ng2009	2.8	2q33.3-q36.3	205.7-235.1			32
Ng2009	2.6	2q21.2-q31.1	146.9-176.3			32
Ng2009	1.4	1p32.2-p31.1	85.8-114.5			32
Ng2009	5.7	5q35.1-q35.3	178.7-208.5			32
Ng2009	8.2	8p22-p12	28.1-56.2			32
Ng2009	10.6	10q26.12-q26.3	145.9-175.0			32
Ng2009	3.4	3p14.1-q13.32	95.9-127.9		+	Eur22
Ng2009	8.2	8p22-p12	28.1-56.2			Eur22
Ng2009	2.8	2q33.3-q36.3	205.7-235.1			Eur22
Ng2009	5.6	5q31.3-35.1	148.9-178.7			Eur22
Ng2009	16.2	16p13.12-q12.2	33.3-66.7			Eur22
Ng2009	3.4	18q22.1-qter	95.9-127.9			Eur22
Ng2009	6.3	6p21.31-p12.1	56.0-84.0			Eur22
Lewis2003	2.5	2p12-q22.1	101.6–128.4	+		20
Lewis2003	5.5	5q23.2-q34	131.5–164.2	+		20
Lewis2003	3.2	3p25.3-p22.1	32.4–63.1	+		20
Lewis2003	11.5	11q22.3-q24.1	99.0–123.0	+		20
Lewis2003	6.1	6pter-p22.3	0–32.6	+		20
Lewis2003	2.6	2q22.1-q23.3	128.4–154.5	+		20
Lewis2003	1.6	1p13.3-q23.3	142.2–170.8	+		20
Lewis2003	22.1	22pter-q12.3	0–33.8	+		20
Lewis2003	8.2	8p22-p21.1	27.4–55.0	+		20
Lewis2003	6.2	6p22.3-p21.1	32.6–65.1	+		20
Lewis2003	20.2	20p12.3-p11	21.2–47.5	+		20
Lewis2003	14.1	14pter-q13.1	0–40.1	+		20
Lewis2003	16.2	16p13-q12.2	32.1–67.6		+	20
Lewis2003	18.4	18q22.1-qter	96.5–126.0		+	20
Lewis2003	10.1	10pter-p14	0–29.2		+	20
Lewis2003	1.7	1q23.3-q31.1	170.8–201.6		+	20
Lewis2003	15.3	15q21.3-q26.1	52.3–85.6		+	20
Lewis2003	6.4	6q15-q23.2	99.0–131.1		+	20
Lewis2003	17.3	17q21.33-q24.3	63.6–94.0		+	20

Table 4. The top 43 candidate genes for schizophrenia based on the Schizophrenia Gene database (SzGene) as of Feb 2010.

Gene	Chr	SzGene #	GA[1]	BR[2]	AE[3]	First reported by	Year
AHI1	6q23	26	++	++	n	Amann-Zalcenstein	2006
AKT1	14q32	20	+++	+	-	Emamian	2004
APOE	19q13	14	+++	+++	-	Harrington	1995
C6orf217	6q23	30	++	-	n	Amann-Zalcenstein	2006
CCKAR	4p15	11	++	+	n	Tachikawa	2000
COMT	22q11	37	+++	+++	+	Chen	1996
DAO	12q24	41	+++	+++	+	Chumakov	2002
DAOA	13q33	7	+++	+	+	Chumakov	2002
DISC1	1q42	16	+++	+++	+	Wilson-Annan	1997
DRD1	5q35	13	m	+++	n	Nothen	1993
DRD2	11q23	19	+++	+++	+	Comings	1991
DRD4	11p15	12	+++	+++	+	Catalano	1993
DTNBP1	6p22	27	+++	+	-	Straub	2002
GABRB2	5q34	18	+++	+++	-	Lo	2004
GRIK3	1p34	35	+++	+++	n	Begni	2002
GRIN2B	12p13	39	+++	+++	n	Nichiguchi	2000
GWA	11p14	15	m	+	n	O'Donovan	2008
GWA	16p13	24	m	+	n	O'Donovan	2008
GWA	10q26	34	m	+	n	O'Donovan	2008
HIST1H2BJ	6p22	23	++	-	n	Shi	2009
HP	16q22	36	+++	++	+	Lovegrove	1965
HTR2A	13q14	8	+++	+++	-	Arranz	1995
IL1B	2q13	42	++	++	n	Laurent	1997
MDGA1	6p21	10	++	+	n	Kahler	2008
MTHFR	1p36	28	+++	+	n	Arinami	1997
NOTCH4	6p21	3	+++	+	n	Wei	2000
NRG1	8p12	33	+++	+++	+	Stefansson	2002
NRGN	11q24	2	++	+++	+/-	Ruano	2006
OPCML	11q25	38	+	-	n	O'Donovan	2008
PDE4B	1p31	4	+++	+++	+/ -	Pickard	2007
PGBD1	6p22	1	+	-	n	Stefansson	2009
PLXNA2	1q32	17	++	+++	n	Mah	2006
PPP3CC	8p21	40	+	+++	-	Gerber	2003
PRSS16	6p22	22	+	-	n	Stefansson	2009
RELN	7q22	9	++	+++	-	Akahane	2002
RGS4	1q23	31	+++	++	-	Chowdari	2002
RPGRIP1L	16q1	32	+	++	n	O'Donovan	2008
RPP21	6p21	29	+	-	n	ISC	2009
SLC18A1	8p21	43	++	++	n	Bly	2005

Table continues

Gene	Chr	SzGene #	GA[1]	BR[2]	AE[3]	First reported by	Year
SRR	17p13	21	++	++	-	Yamada	2005
TCF4	18q21	5	+	-	n	Vincent	1999
TPH1	11p15	6	+++	+++	n	Paik	2000
ZNF804A	2q32	25	+	-	+	O'Donovan	2008

[1] Association studies, evidence from meta-analyses only: m, 1 positive report: +, 2-3 positive reports: ++, >3 positive reports: +++

[2] Biological relevance, no evidence: -, weak: +, some: ++, strong+++

[3] Expression studies: n: not reported, +: up regulation, -: down regulation, +/-: both up and dowm regulation reported

2.5 The DISC1 pathway in the genetic etiology of schizophrenia

*Disrupted in Schizophrenia 1 (DISC1) i*s one of the most promising candidate genes for schizophrenia with abundant supporting evidence from association, linkage and expression studies and animal models. In addition to *DISC1* itself, a number of genes encoding proteins that interact with DISC1 have emerged as promising candidate genes for schizophrenia and other major mental illnesses. As a result, the concept of the DISC1 pathway has been proposed. The hypothesis proposes that in addition to the disruption of *DISC1*, the disruption of the pathways DISC1 is involved in increases the risk of mental illness (Millar et al. 2003).

2.5.1 DISC1

DISC1 was identified as disrupted by a balanced translocation [t(1; 11)(q42.1;q14.3)] in a large Scottish family with a high prevalence of schizophrenia, bipolar disorder and recurrent major depression. The translocation segregated with all the previously mentioned mental disorders. Within these families 29 out of total of 87 family members carried the translocation. Of these 29 individuals, 18 were affected with schizophrenia, bipolar disorder or major depression (St Clair, D. M., Blackwood, D., Muir, W., Carothers, A., Walker, M., Spowart, G., Gosden, C., Evans,H.J. 1990, Blackwood et al. 2001).

2.5.1.1 Association and linkage studies

Independent evidence for *DISC1* was found within Finnish cohorts. Initially a strong linkage replication was found at 1q42 with intragenic *DISC1* markers (Ekelund et al. 2000, Ekelund et al. 2001). This locus was further studied in Finnish families ascertained for schizophrenia and an association between allelic haplotypes of *DISC1* and schizophrenia was found (Hennah et al. 2003, Ekelund et al. 2004). The

strongest association was observed between an allelic haplotype of SNPs rs751229 and rs3738401, later named HEP3, and the disorder. This haplotype especially associated with the disorder in males. Since, many studies worldwide have reported an association between *DISC1* and schizophrenia (Hennah et al. 2003, Hennah et al. 2009b, Hodgkinson et al. 2004, Qu et al. 2007, Chen et al. 2006, Chen et al. 2007, Kim et al. 2008, Mata et al. 2009, Nicodemus et al. 2007, Rastogi et al. 2009, Saetre et al. 2008, Schumacher et al. 2009, Song et al. 2008, Szeszko et al. 2008, Thomson et al. 2005a, Wood et al. 2007, Zhang et al. 2006, Hashimoto et al. 2006, Kockelkorn et al. 2004, Zhang et al. 2005). However, these studies have mostly reported different loci associating with the disorder, and thus no single variant has been found (Figure 7). The effect sizes of the studies have been modest, which is to be expected when studying complex traits. Despite strong evidence for association between *DISC1* and schizophrenia, some studies have failed to detect association between *DISC1* and schizophrenia (Kim et al. 2008, Zhang et al. 2005, Sanders et al. 2008, Devon et al. 2001, Fallin et al. 2005).

In addition to the strong evidence for *DISC1* in association studies, variants in *DISC1* have displayed evidence for association with schizophrenia related endophenotypes. In Finnish families, *DISC1's* HEP3 haplotype associates with visual working memory among males (Hennah et al. 2005). Other variants in *DISC1* have displayed association with: the P300 event related potential (Blackwood et al. 2001), cognitive aging (Thomson et al. 2005a), attention (Liu et al. 2006) and sociability (Li et al. 2007b). In the general population, *DISC1* has displayed evidence of association with anhedonia psychosis proneness measures (Tomppo et al. 2009b). *DISC1* seems to have an impact on brain physiology by associating with reduced gray matter volume in several brain regions, especially in the dorsolateral prefrontal cortex (Cannon et al. 2005). It also affects hippocampal structure and function (Callicott et al. 2005).

Figure 7. The schematic describes the associated loci within the DISC1-TSNAX region to date. Only association studies of schizophrenia have been included.

DISC1 seems to be involved in the etiology of other major mental illnesses as well as some other neurodevelopmental disorders. Associations of *DISC1* with both bipolar disorder and schizoaffective disorder were reported in the Scottish population in the year 2005 (Thomson et al. 2005b). In 2006, an association between *DISC1* and major depression was reported (Hashimoto et al. 2006). *DISC1* displays evidence for association with autism and Asperger's syndrome in Finnish families (Kilpinen et al. 2008). Just recently, associations between *DISC1* and anxiety (Harris et al. 2010) and chronic fatigue syndrome (Fukuda et al. 2010) have been reported.

2.5.1.2 DISC1 protein structure

The alternative DISC1 transcripts give rise to four predicted protein isoforms in humans. The full-length protein isoform comprises 845 amino-acid residues (~100 kDA) . The full-length human DISC1 protein is predicted to consist of an N-terminal region, often referred to as the globular 'head' domain (encoded by exons 1 and 2) and an alpha-helical coiled–coil-containing C-terminal region (encoded by exons 3–13)(Millar et al. 2000, Millar et al. 2001). The experimental three-dimensional structure and biophysical characterization of the encoded protein is still lacking (Chubb et al. 2008).

2.5.1.3 Expression

DISC1 is expressed in many tissues around the human body including the central nervous system, heart, placenta, kidneys and pancreas (Millar et al. 2000, James et al. 2004). The expression levels of DISC1 vary greatly throughout development.

Disc1 is actively expressed throughout mouse brain development in neuronal cells. It is expressed at a high level in the hippocampus and transiently in thalamus (Austin et al. 2004). The expression of the Disc1 100kDa isoform is the most active during the embryonic day 13.5 and the postnatal day 35. The days correspond to the time points of active neurogenesis and onset of puberty in human development (Schurov et al. 2004). Recently, Mao and colleagues reported that Disc1 is also highly expressed in the embryonic ventricular/subventricular zones of the cortex where neural progenitor cells reside (Mao et al. 2009, Brandon et al. 2009). The knock-down of Disc1 on embryonic day 13 leads to decreased neural progenitor cell proliferation and premature neuronal differentiation in the developing cortex (Kamiya et al. 2005).

In human adult brain tissue (post mortem), DISC1 is expressed in the dentate gyrus in the hippocampus and temporal and parahippocampal cortex (James et al. 2004, Lipska et al. 2006, Miyoshi et al. 2003). In studies using mice and monkeys, DISC1 is expressed widely in the cerebellum, cerebral cortex, ammon's horn, olfactory bulbs, paraventricluar and arcuate nuclei, and amygdala (Schurov et al. 2004, Miyoshi et al. 2003, Ozeki et al. 2003, Austin et al. 2003, Ma et al. 2002). Expression in the hippocampus is apparent in all species studied (Harrison 2004).

In immature and proliferating neurons, cells DISC1 is expressed in the centrosomes, mitochondria and nuclei of cell bodies. In the nucleus, DISC1 interacts with the dynein motor complex and ATF4/PML transcriptional machinery (Chubb et al. 2008, Jaaro-Peled et al. 2009). In mature neurons DISC1 is mainly found in the nucleus and the post synaptic density. In the latter it interacts with PDE4B and is suggested to be involved in the regulation of synaptic plasticity (Camargo et al. 2007) (Jaaro-Peled et al. 2009). DISC1 is also expressed in neurites (Chubb et al. 2008). Within hippocampal neurons, DISC1 is expressed within growth cones (Chubb et al. 2008).

It has been suggested that since DISC1 localizes in multiple cell compartments and interacts with different proteins at different locations, it might have different roles in different cell compartments. Furthermore, different DISC1 isoforms might interact with different proteins as a result of preferential localization in a particular sub-cellular compartment (Chubb et al. 2008).

2.5.1.4 Disc1 mouse models

Developing mouse models for psychiatric disorders is difficult, because phenotypic assessment is challenging and the disorders are heterogeneous in etiology and

patophysiology. Some key features of schizophrenia such as hallucinations might be human-specific. Even though no schizophrenic mouse exists, several schizophrenia-related symptoms and behaviors can be studied in mice. Such phenotypes include hyperresponsitivity to novelty, hypersensitivity to psychostimulants and working memory deficits (Desbonnet et al. 2009). Several anatomical and physiological features can also be studied (Desbonnet et al. 2009).

Numerous *Disc1* knockout and transgenic mice have been studied to date. Mice of the 129S9 Sv/Ev strain have been shown to carry a deletion polymorphism in exon 6 of *Disc1*. The deletion introduces a termination codon in exon 7 stopping full-length Disc1 production. The mice display working memory deficits (Koike et al. 2006). *Disc1* mice with chemical mutagenesis (ENU, ethyl nitrosurea alkylation) on exon express impairment in prepulse inhibition and latent inhibition with enhanced locomotor responsitivity to novelty (Clapcote et al. 2007). Transgenic mice expressing a dominant-negative form of Disc1 have enlarged lateral ventricles and enhanced locomotor responsitivity (Hikida et al. 2007). Working memory deficits have also been reported in mice with inducible expression of the Disc1 C-terminal fragment in the early postnatal period (Li et al. 2007b).

2.5.2 The DISC1 pathway

DISC1 is a multifunctional anchoring molecule that regulates interacting proteins in different subcellular compartments (Chubb et al. 2008, Jaaro-Peled et al. 2009, Ishizuka et al. 2006). A large number of proteins that bind to the DISC1 protein have been recognized through protein complex (co-) immunoprecipitation and yeast two-hybridization (Millar et al. 2005, Millar et al. 2003, Miyoshi et al. 2003, Ozeki et al. 2003, Camargo et al. 2007, Miyoshi et al. 2004, Morris et al. 2003, Brandon et al. 2004, Taya et al. 2007, Shinoda et al. 2007). Ingenuity Pathway Analysis Tool reports 135 proteins that directly interact with the DISC1 protein (Feb 2010, http://www.ingenuity.com/). DISC1 and the binding proteins are mainly involved in functions related to the cytoskeleton, cell cycle, signal transduction, intracellular transport / exocytosis, Golgi and central nervous system development (Chubb et al. 2008). Table 5 summarizes the DISC1 pathway genes that have been studied for association with schizophrenia. The following section describes the functions of the key DISC1 binding partners and how they are thought to contribute to the etiology of schizophrenia.

PDE4B was first connected with schizophrenia when found to be disrupted by a balanced translocation between chromosomes 1 and 16 in a Scottish pedigree. This translocation segregated with schizophrenia (Millar et al. 2005). Since then, several reports have implicated associations between *PDE4B* and schizophrenia (Millar et al. 2005, Pickard et al. 2007, Fatemi et al. 2008a, Numata et al. 2008, Kahler et al. 2010, Tomppo et al. 2009a). PDE4B belongs to a large protein family with other PDE4s (PDE4A-PDE4D). Human PDE4B expresses three isoforms. The protein binds to DISC1 in a cAMP dependent manner and catalyzes cAMP. PDE4B is a

target of prototypical antidepressant rolipram, which inhibits its cAMP catalyzing activity (Houslay et al. 2003). Mice deficient in *Pde4b* or *Pde4d* behave as if they were treated with antidepressants by having reduced motility in a forced swimming task (Houslay et al. 2003). PDE4s are homologous to the Drosophila Melanogaster's learning and memory gene *Dunce*. Mutations in this gene cause learning and memory deficits (Davis 1996). Moreover, *Dunce* mutants have altered axonal growth cone motility, neuronal function and synaptic plasticity (Kim et al. 1996, Delgado et al. 1998, Lee et al. 2000).

The homologous *NDE1* and *NDEL1* are candidates for schizophrenia due to their involvement in neuronal migration and neuronal outgrowth and binding to DISC1 ((Hayashi et al. 2005, Sasaki et al. 2005)). Expression levels of NDEL1 are reduced in brain tissue among individuals with schizophrenia (Lipska et al. 2006). Previously, *NDE1* and *NDEL1* were linked to early age neuronal development in mice (Brandon et al. 2004, Sasaki et al. 2005, Schaar 2004). However, recent findings support a wider role for *NDEL1* in adult neurogenesis in mice along with *DISC1* (Duan et al. 2007). Both *NDE1* and *NDEL1* have been implicated in the etiology of schizophrenia in association studies (Tomppo et al. 2009a, Hennah et al. 2007, Burdick et al. 2008).

NDE1 and NDEL1 interact with DISC1 and another DISC1 binding partner LIS1 (Brandon et al. 2004). Disruption of LIS1 may lead to lissencephaly, a difficult neurodevelopmental condition marked by severe brain malformation (Kato et al. 2003, Cardoso et al. 2000, Lo Nigro et al. 1997). Mutations in one copy of the *PAFAH1B1* (*LIS1)* gene cause deficits in cortical layering, neuronal migration and neuroblast proliferation in mice (Hirotsune et al. 1998, Gambello et al. 2003). Deficits in one copy of *Pafah1b1* also result in aberrant neurogenesis in adult mice hippocampus (Wang et al. 2007). Therefore, this gene is also involved in neuronal development. Expression levels of LIS1 are reduced among schizophrenia patient (Lipska et al. 2006).

GSK3β has been implicated in the proliferation of embryonic and adult neural progenitor cells through the GSK3β/β-catenin pathway. In neuronal progenitor cells, DICS1 interacts directly with GSK3β, which reduces the phosphorylation of β-catenin and increases its stability. Consequently, the Wnt signaling pathway is activated (Jaaro-Peled et al. 2009, Mao et al. 2009). Two studies have reported association between *GSK3β* and schizophrenia (Souza et al. 2008, Scassellati et al. 2004). Interestingly, GSK3β activity is affected by many antipsychotic drugs and lithium (a mood stabilizer). Beaulieu and colleagues have implicated *GSK3β* in serotonin homeostasis and suggested that GSK3β signaling is an important pathway through which serotonin deficiency causes abnormal behavior (Ming et al. 2009, Beaulieu et al. 2008).

FEZ1 associated with the disorder in the Japanese population but attempts to replicate this finding have not succeeded (Yamada et al. 2004, Koga et al. 2007,

Hodgkinson et al. 2007). However, expression and mouse studies support the involvement of *FEZ1* in the etiology of schizophrenia. Reduced Fez1 expression in rat hippocampal neurons results in neuronal deficits, such as defects in axonal outgrowth. (Rastogi et al. 2009, Koga et al. 2007, Hodgkinson et al. 2007).

Interaction between DBZ and DISC1 has also been suggested to be involved in neuronal outgrowth (Hattori et al. 2007). However, no evidence of association for the involvement of this gene in the etiology of schizophrenia has been reported to date (Chubb et al. 2008, Jaaro-Peled et al. 2009).

ATF4 and ATF5, which interact with nuclear DISC1, are key regulators of the stress response in neurons. Thus, together with *DISC1* they are hypothesized to play roles in the response to environmental factors relevant to schizophrenia, such as birth hypoxia and congenital infections (Jaaro-Peled et al. 2009). Qu and colleagues have reported association between *ATF4* variants and schizophrenia, but additional evidence for this gene is still lacking (Qu et al. 2008). Positive association findings have been also reported for *PCNT* (Numata et al. 2009a, Anitha et al. 2009).

A number of DISC1 binding partners have been along with *DISC1* implicated also in other major mental illnesses and in autism (Braun et al. 2007, Kahler et al. 2010, Numata et al. 2009a, Shifman et al. 2008, Heck et al. 2008, Calboli et al. 2010, Numata et al. 2009b). This implies that these disorders might share a common etiologic component such as neuronal development (Chubb et al. 2008, Brandon et al. 2009).

Table 5. The table summarizes the DISC1 binding partners that have been studied for their involvement in the genetic liability for schizophrenia. For the genes displaying positive evidence for involvement in the etiology of schizophrenia, the first publication is implicated.

	Evidence for involvement if the etiology of schizophrenia					Other mental illnesses
Gene	GA	AE	AM	Other	Reported	
ATF4	+	n	n		Qu 2008	
ATF5	n	n	n			
CIT	n	n	n			
DBZ	n	n	n			
DISC1	+++	+	+	Translocation, linkage	Millar 2000	BPD AD MD ANX SZ-A SZ-S CFS
DPYSL2	++	n	n		Nataka 2003	
FEZ1	+	-	+		Yamada 2004	
GRB2	n	n	n			
GSK3B	++	n	n		Souza 2008	
KIF5A	n	n	n			
LIS1	n	+	+		Lipska 2006	
MAP1A	n	n	n			
MIPT3	n	n	+			
MPPED1	n	n	n			
NDE1	+	n	n	CNV, linkage	Hennah 2007	
NDEL1	++	+	n		Burdick 2008	
PCNT	++	n	n		Numata 2009	
PDE4B	+++	+	+	Translocation	Millar 2005	MD AD
PDE4D	+	n	n		Tomppo 2009	ANX
SPARCL1	+	n	n		Kähler 2008	
SPTAN1	n	n	n			
TSNAX	++	n	n			
TUBA1A	n	n	n			
YWHAE	+	-	n		Ikeda 2008	
YWHAG	n	n	n			
YWHAZ	+	n	n		Jia 2004	
ZNF365	n	n	n			

GA: genetic association, n: not reported, +: 1 positive report, ++: 2-3 positive reports, +++: >3 positive reports

AE: altered expression: n: not reported, +: up regulated, -: down regulated,

AM: animal models: n: not reported, +: animal model reported

BPD: bipolar disorder, AD: autism spectrum disorders, ANX: anxiety, SZ-A: schizoaffective disorder, SZ-S: schizophrenia spectrum disorders, MD: major depression, CFS: chronic fatigue syndrome

3 Aims of the study

The aim of the present study was to study the role of the DISC1 pathway in the genetic backgrounds of schizophrenia and related traits in the Finnish population. The DISC1 pathway refers to the *DISC1* gene and the other genes encoding proteins that interact with DISC1.

The following specific aims were addressed in the study:

- In Study I, the aim was to recognize new schizophrenia susceptibility loci by utilizing schizophrenia genome-wide linkage data and conditioning it by *DISC1* risk variants previously recognized in the same sample. A follow up was also carried out by mapping the association of the recognized loci with schizophrenia and endophenotypes for schizophrenia.
- In Study II, the aim was to study 11 additional DISC1 pathway genes for association with schizophrenia and endophenotypes of schizophrenia.
- Study III aimed to replicate a previously reported interplay between variants in *DISC1* and to study the impact of *DISC1* on psychosis proneness in the general, healthy population.
- In Study IV, the aim was to recognize new schizophrenia susceptibility loci by using psychosis proneness measures, genome-wide SNP data and a *DISC1*-conditioned approach based on the findings from Study III.

4 Materials and methods

4.1 Table of methods used

A description of the methods used in this study can be found in the original publications accompanying this thesis, as described in Table 6.

Table 6. Table of the methods used.

Material or Method	Original Publication
Study sample	
Finnish Schizophrenia Family Sample	I, II
Quantitative trait sub-sample	I, II
Control Sample	I, II
Northern Finland Birth Cohort 1966 (NFBC66)	III, IV
Phenotype Methods	
DSM-IV Concensus Diagnosis	I, II
Quantitative Neurocognitive Traits	I, II
Psychosis Proneness Scales	III, IV
Statistical Methods	
SNP Selection	I, II, III
Genotype Quality Controls	I, II, III, IV
SNP Association	I, II, III, IV
Haplotype Association	I, II
Bonferroni Correction	III
Replication	II
Permutations	I, II, IV
Haplotype construction	I, II
Quantitative Trait Association	I, II, III, IV
Candidate Genes	I, II, III
Genome-wide Association	IV
Genome-wide Linkage	I
Laboratory Methods	
Genotyping	I, II, III, IV
Sequenom	I, II, III
Taqman	III
Illumina	IV

4.2 Study samples

4.2.1 Finnish schizophrenia family sample

The Finnish schizophrenia family sample is a large sample collection used in many studies of schizophrenia in the Finnish population. Among other studies, this same sample has been previously used in genome-wide linkage and association studies of schizophrenia (Hennah et al. 2003, Ekelund et al. 2000, Ekelund et al. 2001, Ekelund et al. 2004, Tomppo et al. 2009a, Hennah et al. 2007, Wedenoja et al. 2007, Paunio et al. 2001, Hovatta et al. 1994, Hovatta et al. 1998, Hovatta et al. 1999, Turunen et al. 2007, Pietilainen et al. 2009). This family sample has been used in Studies I and II. In Study I the whole sample was analyzed at once (476 families). In the Study II, the family sample was randomly split into two non-overlapping sub-samples to be analyzed as exploratory (171 families) and replication data sets (305 families).

The collection of families was started in 1988 by the National Public Health Institute (since 2009 the National Institute for Health and Welfare) and the sample collection method has not changed in the years since. The individuals with schizophrenia (probands) are identified from three nationwide data registers of: the hospital discharge, disability pension, and reimbursed medication (Lichtermann et al. 1998). The first-degree family members of each proband were thereafter identified through the Population Register Centre, enabling the construction of pedigrees. Personal data recorded in the Population Information System are maintained by the Population Register Centre, and local register offices record personal identity codes, family relations and date of birth and death (if applicable). The registers have allowed for the identification of 33,371 individuals born 1940-1976 who have been diagnosed with schizophrenia (according to the ICD-8, DSM-III-R, or ICD-10 classifications) in the years 1969-1998 (Lichtermann et al. 1998). Family information has been collected from the Finnish population register and pedigrees have been constructed. The sample now includes 476 nuclear families with a total of 2756 individuals, of which 1857 have been genotyped. The lifetime diagnosis for each case in the study sample has been evaluated according to DSM-IV criteria independently by two psychiatrists **(*American Psychiatric Association, Diagnostic and Statistcal Manual of Mental Disorders,* 1994)**. Concordance between the two psychiatrists is high (kappa values range from 95% to 99%, depending on the liability class). In the case of a discrepancy, a third psychiatrist assessed lifetime diagnosis, and the consensus best-estimate lifetime diagnosis was made. A schematic of the collection is given in Figure 8.

In addition to the schizophrenia proband, family members with other psychiatric illnesses were also identified. It was therefore possible to define affection status with increasingly inclusive liability classes (LC). The LC1 consists of individuals

diagnosed with schizophrenia (N = 651). LC2 adds individuals diagnosed with schizoaffective disorder to the sample (N = 132); LC3 individuals affected with schizophrenia spectrum disorders (schizoid, schizotypal and paranoid personality disorders, schizophreniform, delusional and brief psychotic disorder, and psychosis not otherwise specified (N = 103); and LC4 individuals with bipolar affective disorder or major depression with and without the presence of psychosis (N = 83). In Study I, the widest definition of affection status (LC4) was used in order to include all possible information afforded by the sample. In Study II, we restricted our end-state diagnosis phenotype to LC3 due to increasing evidence of genetic liability: among families ascertained for schizophrenia the genetic liability for disorders in both LC2 and LC3 is increased (Tienari et al. 2003), whereas the disorders in LC4—despite having some overlap in genetic risk—display similar genetic liability as the general population (Tienari et al. 2003, Blackwood et al. 2007, Shifman et al. 2004).

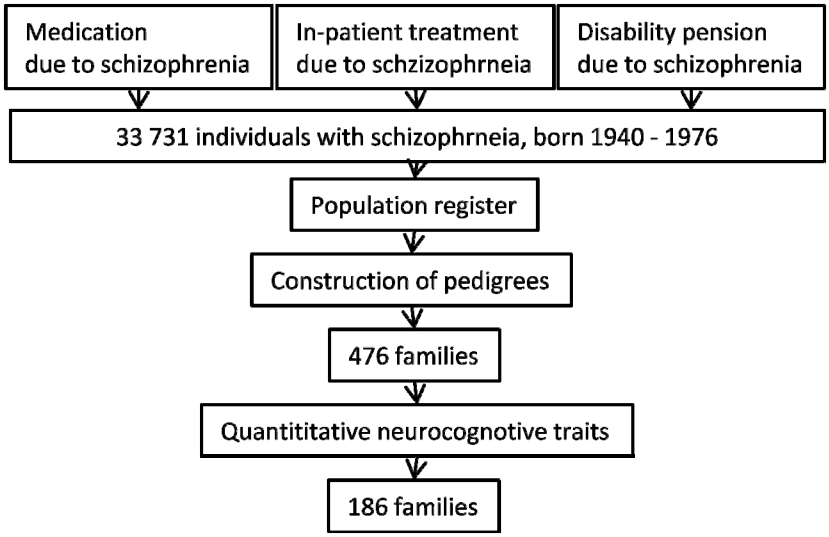


Figure 8. Schizophrenia family sample collection.

4.2.2 Neurocognitive quantitative trait sub-sample

For a sub-sample of 186 families (746 individuals), a neuropsychological test battery was administered (Tuulio-Henriksson et al. 2002). The tests were administered in a fixed order by experienced psychologists and specially trained psychiatric nurses. The test scoring was performed by experienced psychologists. The following variables were used in the analyses.

First, from the Wechsler Memory Scale—Revised (WMS-R) (Wechsler 1987) we included Verbal and Visual Span forward subtests as measures of auditory and

visual attention, respectively (Study II). The respective backward condition of these WMS-R subtests was used as measures of verbal (Study II) and visual working memory (Studies I and II). Second, from the California Verbal Learning Test (CVLT) (Delis, D.C., Kramer, J.H., Kaplan, E. and Ober,B.A. 1987), total recall from trials 1–5 were used as a measure of learning (Study II). The other included CVLT variables were semantic clustering as a measure of learning strategy and short-delay and long-delay recall (Study II). Third, from the Wechsler Adult Intelligence Scale—Revised (WAIS-R)(Wechsler 1981), the Vocabulary subtest was included as an estimate of basic ability, and the Digit Symbol subtest was included as a measure of information processing speed (Study II). The selected traits are either direct measures of learning and memory—such as short-delay memory, long-delay memory, and verbal learning—or highly relevant to learning process, such as auditory attention, visual working memory, verbal working memory, verbal attention, and semantic clustering. The test scores were normally distributed in our sample.

4.2.3 Control sample

The control sample used in studies I and II includes 57 anonymous Finnish families that all included three family members: father, mother and their child. No phenotype data was available for these families. This sample represents a random sample of the Finnish population. In Study I this control sample was used to derive the unbiased SNP and haplotype frequencies in the study sample. This sample was also included in the SNP and haplotype association analyses in study I and in SNP association analyses in Study II in order to maximize the number of unaffected individuals for calculations of expected allele frequencies. Parents of these families were used as control samples in study II.

4.2.4 Northern Finland Birth Cohort 1966

The Northern Finland Birth Cohort 1966 was used in Studies III and IV. The study was started in 1965 in the two northernmost provinces in Finland: Oulu and Lapland. Data on the individuals born into this cohort and their parents were collected from the 24th gestational week. The cohort included 12 055 mothers and they had 12 068 deliveries (13 women delivered twice). The cases belonging to the survey period were determined by the calculated term. The study population comprised 96.3% of all births in 1966 in that area. Altogether, 12 231 children were born into the cohort and 12 058 of them were live born (Rantakallio 1969). The original cohort data have been supplemented by data collected by postal questionnaires at the ages of 1, 14, and 31 years and by various hospital records and national register data. Participants underwent a physical examination at age 31. The data analyzed in this study were included in a questionnaire given to individuals at the 31-year follow-up. A total of 4651 individuals completed the questionnaire and provided a DNA sample and written informed consent; this represented 40.3% of the 11 541 individuals who could be contacted at age 31 and 77.1% of the 6033

individuals who took part in the follow-up study at age 31. Of the 4651 individuals, 55.7% were female and 44.3% were male (Tomppo et al. 2009b).

The scales included in Study III were the Perceptual Aberration Scale (PER) (Chapman et al. 1978),Revised Social Anhedonia Scale (RSAS) (L. Chapman, PhD, unpublished test, 1982) (Chapman et al. 1976), Revised Physical Anhedonia Scale (RPAS) (L. Chapman, unpublished test, 1978) (Chapman et al. 1976) and Schizoidia Scale (SCHD) by Golden and Meehl (Golden et al. 1979). These scales were selected to assess normal properties that correspond to positive and negative aspects of psychotic disorders. The scales were translated into Finnish by a native speaker and were then professionally back translated into English and corrected (Ekelund et al. 1999). The scale items were mixed into a single pen-and-paper questionnaire with items from other scales and were tested in a sample of employees from the National Public Health Institute.

In Study IV, we looked for association at the genome-wide level using the RSAS and RPAS scales, because they showed association with *DISC1* (Tomppo et al. 2009b). The sample was stratified based on individuals' *DISC1* variant profiles. The number of individuals carrying the *DISC1* risk and protective variants were 3049 and 962, respectively. The remaining individuals that did not carry either of these variants comprised a neutral group (N = 505).

The psychometric properties of these scales in this specific setting have been previously reported (Miettunen et al. 2004, Miettunen et al. submitted). Recent psychometric studies have shown that the dimensions of schizotypy measured by the RSAS and RPAS scales are not interchangeable indicators of a single taxon as originally proposed by Meehl (Meehl 1962). Instead they represent different underlying latent traits, namely perceptual aberration as opposed to social anhedonia (Horan et al. 2004). Originally these two scales, but not physical anhedonia, were considered valid indicators for future onset of psychotic disorders (Chapman et al. 1994). Further work on the same longitudinal sample, reviewed by Blanchard et al (Blanchard et al. 2000), showed that after controlling for the effects of the other psychosis proneness measures social anhedonia independently and most clearly predicted later schizophrenia-spectrum personality disorders, social dysfunction, and poor quality of relationships. However, in a recent report by Fonseca-Perdrero and colleagues both physical anhedonia and social anhedonia were shown to be adequate indicators for later schizophrenia spectrum disorders (Fonseca-Pedrero et al. 2009). In addition, a recent report by Horan and colleagues (Horan et al. 2008) showed that along with Social Anhedonia Scale, Physical Anhedonia Scale can be used as a psychosis vulnerability indicator. In that study, patients showed steady elevations on the Physical Anhedonia Scale across time and clinical state, consistent with a stable vulnerability indicator.

Table 7. Descriptive statistics on the outcome measures in the NFBC66 sample.

	Scores of the combined sample/women/men			
	PER	RSAS	RPAS	SCHD
N	4651	4651	4651	4651
Mean	2.33/2.54/2.06	9.39/8.08/11.03	15.02/12.70/17.94	2.54/2.77/2.25
SD	3.17/3.27/3.03	5.44/4.70/5.84	6.98/5.85/7.20	1.41/1.36/1.40
Range	0-35 (all)	0-40 (all)	0-61 (all)	0-7 (all)

Abbreviations: PER, Perceptual Aberration Scale; RPAS, Revised Physical Anhedonia Scale; RSAS, Revised Social Anhedonia Scale; SCHD, Schizoidia Scale by Golden and Meehl.

Table 8. Descriptive statistics on the outcome measures after the DISC1-based stratification in the NFBC66 sample in Study IV.

	Scores of the combined sample					
Variable	RSAS			RPAS		
Model	Risk	Protective	Neutral	Risk	Protective	Neutral
N	3049	962	505	3049	962	505
Mean	9.64	8.97	9.52	15.28	14.53	15.04
SD	5.51	5.51	5.62	7.12	6.95	7.12
95% CI of mean	9.44-9.84	8.62-9.33	9.02-10.03	15.02-15.53	14.08-14.97	14.40-15.68
Range		0-40			0-61	

Abbreviations: RPAS, Revised Physical Anhedonia Scale; RSAS, Revised Social Anhedonia Scale.

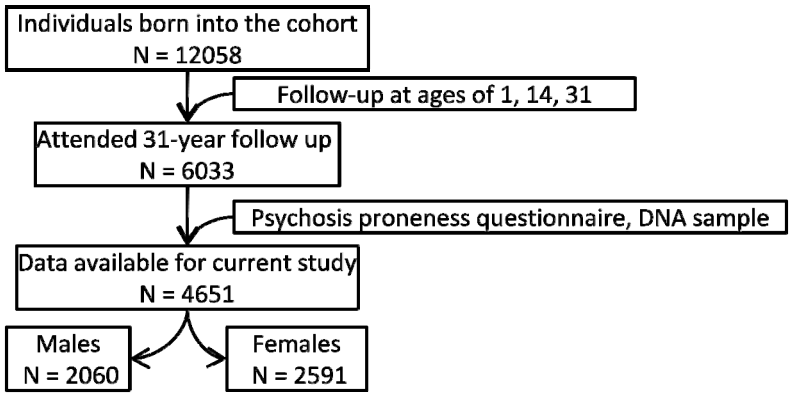


Figure 9. Northern Finland Birth Cohort 1966 sample collection (NFBC66).

4.3 Statistical methods

4.3.1 Linkage analyses

A genome-wide linkage analysis was performed on two stratified samples (carriers of the *DISC1* risk haplotype HEP3: 145 families, non-carriers of the *DISC1* risk haplotype HEP3: 313 families) and the combined data set using genotypes from 443 microsatellite markers. The microsatellites were previously genotyped and analyzed by our research group (Ekelund et al. 2000, Ekelund et al. 2001, Ekelund et al. 2004, Paunio et al. 2001, Hovatta et al. 1999). Two-point linkage analysis was carried out using the heterogeneity model in the MLINK program (Lathrop et al. 1985). The scan was performed in all three samples for all four increasingly inclusive LCs and using both dominant and recessive models. Testing all of these models in each sample meant that 24 genome scans were being tested. Therefore, simulation of the data was essential to derive the significance of any results in the face of multiple testing and the additional biasing presented by the conditioning on *DISC1*. The simulation of linkage significance was carried out by randomly reassigning genotypes to individuals at frequencies identical to the original analysis, to create 100 random replicates of the sample. Across all eight models for the three samples (un-stratified, HEP+, HEP-), only LOD-scores >3 met the 0.05 level of significance.

4.3.2 Association analyses

4.3.2.1 SNP selection

For the studies of association between the DISC1 pathway genes and schizophrenia (Studies I and II), the SNPs were selected from the international HapMap project database builds # 15 (study I) and #16, phase 1 (study II) (Consortium 2003). The LD structure of candidate genes was defined using all the SNPs with a minor allele frequency > 5% in the population of European descent (CEU, Utah residents with ancestry from Northern and Western Europe). We used the Haploview program and the solid spine of LD method for defining the LD block structure (D' > 0.8). The method searches for a "spine" of strong LD running from one marker to another in which the first and last markers in a block are in strong LD with all intermediate markers while intermediate markers are not necessarily in LD with each other (Barrett et al. 2005). LD blocks with a Hedricks multiallelic D' $\geq$ 0.9 were combined. The method treats each block as a single marker and estimates the LD between the blocks (Hedrick 1987). Haplotype tagging SNPs were selected to obtain optimal coverage of the genes. Additional SNPs from the HapMap and Perlegen (Hinds et al. 2005) databases were selected to provide coverage if genotypes of tagging SNPs were not of sufficiently high quality.

In study III, the SNPs selection was based on previous study of *DISC1* (Hennah et al. 2009b). In that study the methodology for SNP selection was the same as

described above. Study III included SNPs that best tagged the *DISC1* gene and SNPs having previously displayed association with schizophrenia.

In Study IV, the sample was genotyped using the genome-wide Illumina HumanHap 370K SNP array.

4.3.2.2 Haplotype estimation

To perform haplotype analyses in the family sample (Studies I and II), we identified haplotype blocks according to the LD structure defined by Haploview, as described above. In genes *NDE1*, *NDEL1*, *TRAF3IP1*, *ATF4*, *ATF5*, *MAP1A*, *TUBA1A*, *FEZ1* and *PCNT* only one haplotype block for each gene was present. *PAFAH1B1*, *PDE4B* and *PDE4D* included 2, 10 and 17 LD blocks, respectively. In *ATF4*, *PDE4B* and *PDE4D* we were able to tag 0, 8 and 16 blocks respectively. All the blocks were tagged in other genes,.The LD structure in our sample correlated well with the LD structure defined using the HapMap CEU population. However, for *NDE1* and *NDEL1* the LD pattern in the CEU population predicted two LD blocks, while only one block was predicted according to our sample. We analyzed the blocks as one.

4.3.2.3 Dichotomous disease phenotype

SNP association analyses in Studies I and II were performed using the Pseudomarker program (Goring et al. 2000). The program performs linkage as well as linkage disequilibrium analyses on samples of mixed form (families and trios being combined here), corrects for the effect of linkage on the association tests and is able to deal with cases where parental genotypes are not known (Goring et al. 2000).

Haplotype association analyses were performed in Study I using the TRANSMIT program (Clayton 1999). It can test for transmission of a haplotype even when the phase is unknown and when parental phenotypes are not complete. The program is able to compensate for the presence of linkage when analyzing family data by calculating a robust variance estimate and by enabling the testing of one offspring per family. However, TRANSMIT does not take controls into account in the analyses.

Haplotype association analyses in study II were performed in a two-stage design using the chi squared test to test each possible haplotype within an LD block against all other haplotypes in a 2x2 table (affected vs. controls). A global test in a 2 x n table was also performed for haplotypes displaying significant association.

In Study I, results were corrected for multiple testing using permutation and Bonferroni corrections. The association tests between affection status and gene variants in Study II were performed using the two-stage study design described above.

4.3.2.4 Quantitative phenotypes

In Studies I and II, quantitative neurocognitive phenotypes (endophenotypes) of schizophrenia were studied. Analyses were performed for the SNPs and haplotypes that displayed significant evidence for association with schizophrenia. The measures used in Studies III and IV were intermediate phenotypes for psychosis.

4.3.2.4.1 Quantitative neurocognitive traits

Analyses were performed using the QTDT program and the orthogonal model (Abecasis et al. 2000a, Abecasis et al. 2000b). Haplotypes were re-coded to form "bi-allelic" markers, so as to test the hypothesized variant against all others combined. Age, sex and affection status according to LC3 (study II) and LC4 (Study I) were used as covariates in the analysis. To achieve empirical results, 100 000 permutation were performed.

4.3.2.4.2 Psychosis proneness phenotypes

In Study III, the analyses were performed using SPSS (SPSS, version 15.0 for Windows; SPSS Inc, Chicago, Illinois). Because all test variables were slightly skewed to the right, logarithmic transformations were performed for each variable. After transformation, the RSAS, RPAS and SCHD were normally distributed. PER could not be normalized because its mode score was 0 (range, 0-35), and thus it was considered non-normally distributed in all analyses.

The association analyses of *DISC1* SNPs with psychosis proneness scales (Study III) were performed using independent samples. The *t*-test was used for the normally distributed traits (RSAS, RPAS, SCHD) and the Mann-Whitney test for PER. Dominant and recessive genetic models were applied in the analyses. All tests were performed for the combined sample and for men and women separately. The 41 SNPs were tested individually for association. To estimate the statistical significance of our findings, the Bonferroni correction was used.

To test our primary hypothesis that the previously reported interplay among SNPs rs1538979, rs821577, and rs82163322 would hold true in our sample, the SNPs were further analyzed dependent on the absence and presence of risk alleles at the other two loci. The multiple minor alleles at these marker loci were analyzed against all others (marker combinations: rs1538979 and rs821577; rs821577 and rs821633; rs1538979 and rs821633; and rs1538979, rs821577, and rs821633). The minor alleles at only one of the three marker loci were analyzed against all others. No P-value correction was performed for these tests because they were strictly hypothesis-based. Effect sizes for the significant variants were estimated using Cohen d' (Cohen 1988).

Statistical tests in the genome-wide association study (Study IV) were performed using the genome-wide association analysis tool set PLINK (version 1.05) (Purcell

et al. 2007). We used linear regression and the additive genetic model to test for association between all the markers and RSAS and RPAS. We used the generally accepted limit of P < 5E-7 for genome-wide significance (Wellcome Trust Case Control Consortium 2007). The analyses were conditioned by *DISC1* variants. All the analyses corrected for sex.

4.4 Laboratory methods

4.4.1 Genotyping methods and quality control

In study I, we used previously genotyped genome-wide linkage data (Ekelund et al. 2001, Ekelund et al. 2004, Paunio et al. 2001). The samples had been previously genotyped by the ABI 377 automated DNA sequencer (Applied Biosystems, Foster City, CA, US). 370 microsatellite markers have been genotyped in these families on the autosomes and chromosome X with an average spacing of 10.5 cM.

In Studies I, II and III, SNP genotyping was performed using the Sequenom platform which is based on laser desorption ionization time-off-flight mass spectrometry (Sequenom Inc, San Diego, CA, US) according to the manufacturer's recommendations (Jurinke et al. 2001). In Study III, one additional marker was genotyped using 5' nucleases method (Taqman protocol) (Applied Biosystems, Foster City, CA, US). SNPs were later rejected if they had a genotyping success rates < 80%. The Hardy-Weinberg disequilibrium test was performed for all SNPs.

In Study IV, the genotyping was performed using the Illumina Infinum Assay and HumanCNV370-Duo marker set. SNPs with success rates < 95 % were excluded from the analyses. Only SNPs with MAF (minor allele frequency) > 0.05 were analyzed. Individuals with genotyping success rates < 95 % were also excluded from the analyses. After quality controls, 331 307 out of a total of 339 054 SNPs and 5251 individuals were included in the analyses. We had phenotypic information available for 4516 individuals of the individuals.

4.5 Ethical consideration

This study has followed the declaration of Helsinki and its amendments in full (World Medical Association. 2001). The study has been proved by Ministry of Social Affairs (Finland) and appropriate institutional review boards.

All the participants in the study have provided their written informed consent.

To ensure the anonymity of the individuals participating in the study, all the individuals have been given a code that is used in all stages of the study and data handling. No data allowing for the identification of study subjects have been used in the study. Only certain researches are allowed to access the sensitive original personal information.

5 Results and discussion

5.1 Conditioned genome-wide linkage study on schizophrenia (Study I)

Since schizophrenia is a complex disorder with multiple genetic factors interacting in the background, we hypothesized that by conditioning our sample on the previously recognized *DISC1* susceptibility variants we would be able to recognize new potential schizophrenia susceptibility loci. We performed a genome-wide linkage study conditioned on the previously recognized allelic haplotype of *DISC1* (HEP3). This haplotype previously displayed evidence for association with schizophrenia and memory functions in the Finnish schizophrenia family sample (Hennah et al. 2003, Hennah et al. 2005).

Linkage with the *DISC1* locus was first detected using 168 families. Due to an increase in sample size since the original study, the present study takes advantage of data from 458 families. We first performed an un-stratified analysis of the new, whole data set to qualify the previous linkage findings. In the unconditioned analyses two genomic loci displayed evidence for linkage (LOD > 3): marker D1S2709 intragenic of *DISC1* and D5S647 on chromosome 5q12.3.

The stratification was based on the HEP3 haplotype. Families with carriers of the HEP3 haplotype were analyzed as one group (N = 145) and non-carriers as another group (N = 313). After conditioning, three loci provided evidence for linkage (LOD > 3): 1q42 (D1S2709; LOD = 3.31, LC3), 10q21 (GATA101E02; LOD = 3.58, LC4) and 16p13 (D16S764; LOD = 3.17, LC1). All three were within the sub-sample of families carrying the *DISC1* risk allele. In addition to these, nine loci displayed suggestive evidence for linkage (LOD > 2) in the stratified analyses. Results are summarized in table 9.

Table 9. HEP3 conditioned linkage scan, the most significant results.

Location	LOD score	Sub-sample	P-value	Candidate genes
1p13.2	2.2	HEP+	0.69	
1q42	3.31	HEP+	0.06	DISC1
2q32.2	2.06	HEP+	0.79	
5q12.3	2.59	HEP–	0.35	
6p21.2	2.16	HEP+	0.71	DTNBP1
7q22	2.25	HEP+	0.64	GRM3, RELN
8p22	2	HEP+	0.83	NRG1
8q24.13–q24.21	2.58	HEP–	0.36	
10q21.3	3.58	HEP+	0.04	
11q22.3	2.8	HEP+	0.22	
12q21.1	2.19	HEP+	0.69	DAO
16p13.11–p12.1	3.17	HEP+	0.08	NDE1

5.1.1 Conclusions

In the conditioned analysis, we detected significant evidence for linkage on chromosome 16p13. In this region, 0.8 Mb from the best marker D16S764, a gene encoding a known DISC1-binding protein, *NDE1*, is located. Interestingly, this locus has also displayed evidence for linkage in Finnish bipolar disorder families (Ekholm et al. 2003), suggesting that the genomic locus might be of importance in the genetic etiology of other mental illnesses as well.

Another marker that displayed significant evidence for linkage, D1S2709, is located within *DISC1*. It was expected that this marker would display a high LOD score in the conditioned analyses, because this marker showed the initial significant linkage in Finnish families and led to the discovery of the HEP3 haplotype, which was used here as the ascertainment criterion. The region where the GATA101E02 marker on chromosome 10q21 is located has previously displayed significant linkage to schizophrenia in Ashkenazi Jews (Fallin et al. 2003) and suggestive evidence in other studies (Levinson et al. 1998, Mowry et al. 2000). Based on the current knowledge, this region does not include any known schizophrenia or DISC1 pathway genes. However, in this region (< 5 kb from the marker) two non-coding genes are located (*HBII-419* and *MIR1254*). It might be of interest to study these RNA genes for their involvement in the etiology of mental illnesses.

Altogether, we detected at least suggestive evidence for linkage (LOD > 2) within 12 genomic regions. Of all these loci, ten are located at or within 50 cM from genomic regions previously identified in the linkage studies of schizophrenia. The ten loci included the genome regions containing the most promising schizophrenia candidate genes at the time: *DTNBP1* (6p21) (Straub et al. 2002), *NRG1* (8p22)

(Stefansson et al. 2002), *GRM3* (Fujii et al. 2003) and *RELN* (7q22) (Impagnatiello et al. 1998). Increasing evidence for these genes supports their roles in the etiology of schizophrenia (Schwab et al. 2009, Harrison et al. 2008). Furthermore, many of these genes are parts of DISC1 related pathways. RELN interacts with DISC1 through ApoER2 (LRP8) and *PAFAH1B1* (LIS1) contributing to neuronal migration (Wynshaw-Boris et al. 2001, Assadi et al. 2003). GRM3 interacts with DISC1 through RANBP9 and DTNBP1 through DMD or UTRN (Ingenuity Pathway Analysis). DISC1 and NRG1 have also been suggested to be functionally convergent (Jaaro-Peled et al. 2009). These observations further strengthen the importance of these observations and suggest that this kind of conditioned approach is a valid tool for the recognition of new susceptibility genes.

5.2 DISC1 pathway candidate gene association studies (Studies I and II)

5.2.1 NDE1 (Study I)

We followed up the linkage on 16p13 near the DISC1 binding partner *NDE1* by genotyping SNP markers spanning the *NDE1*-gene. Seven single nucleotide polymorphisms (SNPs) over 75 kb of the *NDE1* gene were genotyped and association between these SNPs as well as the 4-SNP tag haplotype was analyzed. SNPs rs6498567, rs881803 and the tag haplotype displayed evidence for association with schizophrenia after 100 000 permutations with P-values of 0.0086, 0.021 and 0.0065, respectively. However, none of these remained significant after the Bonferroni correction.

Since *DISC1* had previously displayed sex-dependent effects, we wanted to test whether such effects may also affect the association with the *NDE1*. All the SNPs and the tag-haplotype displayed $P < 0.05$ after 100 000 permutations when the females were analyzed alone. No evidence for association was detected when the transmission to only male offspring was monitored. After Bonferroni correction for the 24 tests performed, the female-specific association to the tag-haplotype (permutated P-value = 0.00046; observed transmissions = 237; expected transmissions = 208) remained significant ($P = 0.011$). The risk allele of the *NDE1* tag-haplotype comprised the CGCC alleles of the SNPs rs4781678, rs2242549, rs881803 and rs2075512, respectively. This haplotype was present in the founders of the schizophrenia families at a frequency of 30 %, whereas in the population control sample its frequency was 19 %. To account for the possible confounding effects linkage might have on the result in the family sample, an additional haplotype association analysis was performed using only one affected offspring per family in the analyses. The tag-haplotype remained significant after this test only among affected females ($P = 0.0024$), but was not significant in the whole sample ($P =$

0.072). However, this might be due to reduction in sample size (N = 1494 individuals compared with 2756 in the whole sample).

To test whether the *NDE1* tag-haplotype explains the observed linkage, an additional linkage analysis was performed. Within the whole sample, the *NDE1* tag-haplotype provided greater evidence for linkage than the microsatellite D16S764 (LOD = 1.00, compared to LOD = 0.78, respectively). However, when the families carrying the *DISC1* risk haplotype HEP3 were analyzed separately, the *NDE1* haplotype provided evidence for linkage with LOD-score of 1.26 while the closest microsatellite D16S764 displayed LOD = 3.17. This suggests that other nearby variants may have also partially contributed to the observed linkage signal.

DISC1 has previously displayed evidence within this same family sample for association with visual working memory, a neurocognitive endophenotype for schizophrenia (Hennah et al. 2005). Because *NDE1* is involved in the same pathway with *DISC1*, we hypothesized that *NDE1* might contribute to the same phenotype. We looked for association between visual working memory and the *NDE1* tag haplotype, which was the variant displaying the most significant evidence for association with affection status. Since measures of neurocognitive quantitative traits were available for only part of the schizophrenia family sample, we first analyzed the association with the end-state diagnosis in order to assess the effect of the reduced sample size (N = 215 families out of 458 families of the complete study sample). In this quantitative trait sub-sample, *NDE1* tag-haplotype displayed P = 0.046 when the whole sample was analyzed and P = 0.016 when only the females were included in the analyses. The *NDE1* tag-haplotype did not provide evidence for association with visual working memory. Permutated P-values for the whole sample, females and males were 0.083, 0.055 and 0.15, respectively.

Table 10: Observed P-values for the SNPs within NDE1 and the NDE1 spanning tag-haplotype. P-values are provided for both association tests between schizophrenia diagnosis and visual working memory.

	Outcome measure	P All	P Males	P Females
Whole schizophrenia family sample				
rs4781678*	Schizophrenia diagnosis	0.051	0.66	0.0052
rs6498567	Schizophrenia diagnosis	0.0086	0.48	0.0024
rs2242549*	Schizophrenia diagnosis	0.27	0.4	0.018
rs881803*	Schizophrenia diagnosis	0.021	0.53	0.0013
rs1050162	Schizophrenia diagnosis	0.17	1	0.014
rs2075512*	Schizophrenia diagnosis	0.078	0.53	0.0044
rs11130	Schizophrenia diagnosis	0.34	0.44	0.023
*Tag haplotype	Schizophrenia diagnosis	0.0065	0.93	0.00046
Quantitative trait sub-sample				
*Tag haplotype	Schizophrenia diagnosis	0.046	0.25	0.016
*Tag haplotype	Visual working memory	0.083	0.15	0.055

5.2.2 Association analysis of 11 DISC1 binding partners (Study II)

We continued to study the DISC1 pathway in the etiology of schizophrenia by studying 11 additional DISC1 binding partners to association with schizophrenia. We analyzed SNP markers and haplotypes for association with schizophrenia using a two-stage study design with random sampling of our original Finnish families. The number of families available for the study was 476. Association analyses between the affection status and SNPs and haplotypes were first performed in an exploratory sample of 171 families. The SNPs and haplotypes displaying evidence for association with P-values <0.05 were reanalyzed in a replication sample set consisting of 305 independent families. Markers and haplotypes passing the replication criteria in the second stage were analyzed with both sample sets combined.

In stage one, 14 SNPs and 19 haplotypes in *NDEL1*, *PDE4B*, *PDE4D*, *PAFAH1B1*, *MAP1A*, and *TRAF3IP1* displayed P-values < 0.05, and thus met our criterion for progressing to stage two. No markers or haplotypes in *FEZ1*, *PCNT*, *TUBA1A*, *ATF4*, and *ATF5* met this criterion (Table 11). In stage two, SNP rs17806986 in *NDEL1* and a *NDEL1* spanning tag haplotype displayed evidence for association with P-values of 0.003 (in stage I P = 0.022) and 0.007 (stage I P = 0.005), respectively. Within *PDE4B*, two allelic haplotypes within the same LD block displayed replicated evidence for association CCC and CTT alleles of SNPs rs4503327, rs2503222 and rs6588186 with P-values of 0.048 (Stage I P = 0.029) and 0.025 (Stage I P = 0.001), respectively. Further, SNP rs7412571 located in the neighboring LD block displayed evidence for association with a P-value of 0.048 (stage I P = 0.028). Within *PDE4D*, replicated association was detected with an allelic haplotype spanning the haplotype of SNPs rs13190249, rs1120303, rs921942, rs10805515 and rs10514862 with P-value of 0.001 (Stage I P = 0.0059). SNP rs1120303 also displayed an independent effect on schizophrenia with P-values of 0.006 and 0.027 in stages II and I, respectively. Markers and haplotypes displaying significant evidence for association in Stage II were tested for their overall significance in the combined sample set. The results of the combined sample are presented in Table 12.

DISC1, *NDE1* and other DISC1 binding partners have displayed gender differences in previous genetic association studies (Hennah et al. 2003, Pickard et al. 2007, Hennah et al. 2007). We tested for any potential gender differences by comparing allelic frequencies of the associating variants between males and females in our sample. We detected no differentiating effects between female and male offspring in the Finnish families.

Some of the affected individuals in the sample are siblings. We wanted to account for the possible confounding effect of linkage by performing the haplotype association analysis by only using one affected offspring per family in the analyses. Three of the four haplotypes remained significantly associated with the following P-

values: *PDE4D* = 0.0055, *NDEL1* = 0.0018, and *PDE4B* protective and risk = 0.0025 and 0.070, respectively.

DISC1 has previously shown association with visual working memory in our family sample (Hennah et al. 2005) and other quantitative neurocognitive traits in other sample sets (Cannon et al. 2005, Blackwood et al. 2001, Thomson et al. 2005a). We wanted to test if our newly identified variants also have similar effects on the traits. In addition to visual working memory, we included several learning- and memory-related variables in the analyses. Due to the smaller size of the neuropsychologically tested sample, we tested all the recognized SNPs and haplotypes in the combined sample only. Of the seven variants, none associated significantly ($P < 0.05$) with any of the nine traits tested.

Table 11: The table summarizes the 11 DISC1 binding partners studied.

		SNPs, P < 0.05		Haplotypes, P < 0.05	
Gene	SNPs	Stage 1	Stage 2	Stage 1	Stage 2
ATF4	-	-	-	-	-
ATF5	4	0	-	0	-
FEZ1	6	0	-	0	-
PCNT	10	0	-	0	-
PAFAH1B1	6	0	-	1	0
MAP1A	5	0	-	1	0
TRAF3IP1	7	2	0	2	0
NDEL1	4	4	1	2	1
PDE4B	34	3	1	5	2
PDE4D	69	5	1	8	1
TUBA-1A	2	0	0	0	-

Table 12: The table summarizes findings in the combined sample for all the SNPs and haplotypes that passed the replication in stage 2.

		SNPs			Haplotypes				
Gene	SNP	P	FA	FC	Global P	Alleles	P	FA	FC
NDEL1	rs17806986	0.0038	0.27	0.37	0.033	CGCG	0.0027	0.12	0.19
Block 1	rs1391768	*nt*	*nt*	*nt*					
	rs1391766	*nt*	*nt*	*nt*					
	rs3817330	*nt*	*nt*	*nt*					
PDE4B	rs4503327	*nt*	*nt*	*nt*	0.006	CCC	0.029	0.28	0.21
Block 4	rs2503222	*nt*	*nt*	*nt*		CTT	0.0022	0.02	0.06
	rs6588186	*nt*	*nt*	*nt*					
PDE4B	rs10158178	*nt*	*nt*	*nt*	*nt*	*nt*			
Block 5	rs7412571	0.018	0.45	0.35					
	rs599235	*nt*	*nt*	*nt*					
	rs2069278	*nt*	*nt*	*nt*					
PDE4D	rs13190249	*nt*	*nt*	*nt*	0.0034	GGACA	0.00084	0.40	0.28
Block 15	rs1120303	0.021	0.13	0.19					
	rs921942	*nt*	*nt*	*nt*					
	rs10805515	*nt*	*nt*	*nt*					
	rs10514862	*nt*	*nt*	*nt*					

nt: not tested

5.2.3 Conclusions

In the present study, we found evidence for significant association between SNP and haplotype variants in *NDE1*, *NDEL1*, *PDE4B* and *PDE4D* and schizophrenia. We found no evidence for association between *ATF4*, *ATF5*, *FEZ1*, *MAP1A*, *PAFAH1B1*, *TRAF3IP1*, *PCNT* or *TUBA1A* and schizophrenia.

PDE4B is currently the most studied of the *DISC1* pathway genes after *DISC1* itself. At present, the SzGene database ranks *PDE4B* as the fourth best candidate gene for schizophrenia based on association evidence (Allen et al. 2008). After the initial report on the connection between *PDE4B* and schizophrenia (Millar et al. 2005), four studies in addition to ours have reported positive association between schizophrenia and the gene (Pickard et al. 2007, Fatemi et al. 2008a, Numata et al. 2008, Kahler et al. 2010). However, negative association findings have also been reported (Rastogi et al. 2009, Holliday et al. 2009). In the SzGene meta-analysis, SNP rs910694 displayed significant association with schizophrenia. This SNP was not included in our study and other SNPs in the same region did not display evidence for association in our study. However, previous studies by Pickard et al and Fatemi et al reported associating variants at the same loci with which we detected association (Pickard et al. 2007, Numata et al. 2008).

We are the only study reporting association between *PDE4D* and schizophrenia to date. However, *PDE4D* has displayed evidence for association with neuroticism in several reports (Shifman et al. 2008, Heck et al. 2008, Calboli et al. 2010). Neuroticism is a moderately heritable personality trait and is considered to be associated with a risk for developing major depression, anxiety disorders and dementia (Calboli et al. 2010). Like *DISC1, PDE4D* might thus be involved in the etiology of a wide spectrum of mental disorders. Thus, studying the association between *PDE4D* and other mental disorders would be of interest. Further, association between *PDE4D* and schizophrenia has to be verified in other samples and populations.

A haplotype within *NDEL1*, consistent with our finding, has also previously displayed evidence for association with schizophrenia in US Caucasian population (Burdick et al. 2008). Furthermore, significant interplay between *NDEL1, NDE1* and *DISC1* has been reported (Burdick et al. 2008). These findings suggest competitive binding between NDE1 and NDEL1. In other independent studies, SNPs in *NDE1* have not displayed evidence for association with schizophrenia. However, in our study the strongest association was found with haplotypes.

In the present study, we took the novel approach of studying candidate genes for schizophrenia based on information on their interaction with DISC1 and their molecular functions. Even though we did not detect evidence for association with the other candidate genes studied, other groups have reported positive findings between schizophrenia and *PCNT* (Numata et al. 2009a, Anitha et al. 2009) and *FEZ1* (Yamada et al. 2004). However, despite the attempts, other association studies have failed to detect association between *FEZ* and schizophrenia (Rastogi et al. 2009, Koga et al. 2007, Hodgkinson et al. 2007).

DISC1 has previously displayed an association with visual working memory in the same Finnish sample we used for studying *NDE1, NDEL1, PDE4B* and *PDE4D*. However, schizophrenia susceptibility variants within these genes did not display evidence with the tested learning and memory related endophenotypes. It is likely that these genes contribute similarly in the etiology of schizophrenia since these genes are involved in the same intracellular pathways. Thus, is would have been expected to detect an association between these genes and working memory as well. One reason for lack of association might be lack of power due to inadequate sample size.

Our findings support other reports on the DISC1 binding partners, and thus support the involvement of *NDE1, NDEL1, PDE4B* and *PDE4D* in the etiology of schizophrenia.

5.3 *DISC1* and psychosis proneness (Study III)

5.3.1 Exploratory analyses

In Study III, we studied the association between variants in *DISC1* and psychosis proneness measures in the general population. We genotyped 41 SNPs covering the *DISC1-TSNAX* region. All 41 SNPs were tested for association with four variables: Revised Social Anhedonia Scale (RSAS), Revised Physical Anhedonia Scale (RPAS), Perceptual Aberration Scale (PER) and Golden and Meehl Schizoidia Scale (SCHD). Association analyses applied both dominant and recessive genetic models. SNP rs821577 displayed significant association after correcting for multiple testing in the combined sample under the dominant genetic model. Carriers of the minor allele of this SNP had significantly higher scores in RSAS (P = 0.000021) in the combined sample (minor allele frequency, MAF = 0.44). Females contributed more to the statistical significance when gender-specific analysis was performed (P = 0.00019 in females and P = 0.040 in males). Modest uncorrected association showing higher scores in RPAS were detected in the combined (P = 0.021) and female samples (P = 0.0056). SNPs rs11122381 and rs821592 displayed significant recessive effects on RSAS when females were analyzed separately. Carriers of the minor alleles of these SNPs had significantly lower scores in RSAS with P-values of 0.0000038 for rs11122381 and 0.000042 for rs821592. As the threshold to maintain a Type I error rate of $\alpha = 0.05$ after multiple testing correction was uncorrected $P < 0.000053$, these three SNPs remained significant even after correction: marker rs821577 when analyzed in the combined sample and markers rs11122381 and rs821592 when females where analyzed separately. The significant findings are summarized in Figures 10 and 11a.

Even though no other SNPs remained significant after the multiple testing correction, SNP rs821616 that has previously associated with schizophrenia and cognitive aging (Qu et al. 2007, Kim et al. 2008, Thomson et al. 2005a, Callicott et al. 2005) displayed modest uncorrected association with lower scores in SCHD in our sample. P-values in both combined and male samples were 0.022 (MAF = 0.32).

5.3.2 Replication of the previously reported interplay between three SNPs

Our main goal in Study III was to test our a priori hypothesis of interplay between three *DISC1* SNPs. A previous study described a modulation of effect when SNPs rs1538979, rs821577 and rs821633 were reanalyzed conditioned on the genotypes of the other SNPs. These SNPs significantly associated with schizophrenia and bipolar disorder (Hennah et al. 2009b). Since the SNPs were the only SNPs displaying significant interplay in the previous study, we restricted our interplay testing to these SNPs. Significant association was detected again with both RSAS and RPAS, but not with PER or SCHD. When the risk allele of SNP rs821577 was accompanied by the minor (C) allele of SNP rs821633, association with RSAS was detected in the

combined and female samples (P = 0.00054, frequency (f) = 0.468 in the combined sample and P = 0.00097, f = 0.467 in females), with carriers of these alleles having significantly higher scores on the variables). This combination of alleles was also modestly associated with RPAS when females were analyzed separately (P = 0.019) and also approached significance in the combined sample (P = 0.067), again with the carriers having higher scores. When the SNPs were tested conditioned on the absence of the minor alleles of the other two SNPs, the minor allele of SNP rs821633 (f = 0.211) associated significantly with lower scores in both RSAS and RPAS. P-values in the combined sample for RSAS and RPAS were 0.0000022 and 0.0075, respectively. Significant association with RSAS was also detected when females and males were analyzed separately (P = 0.00031 for females, f = 0.220 and P = 0.011 for males, f = 0.199). Furthermore, RPAS associated significantly with this marker in females (P = 0.0086). The significant findings are summarized in Figures 10, 11b and 11c.

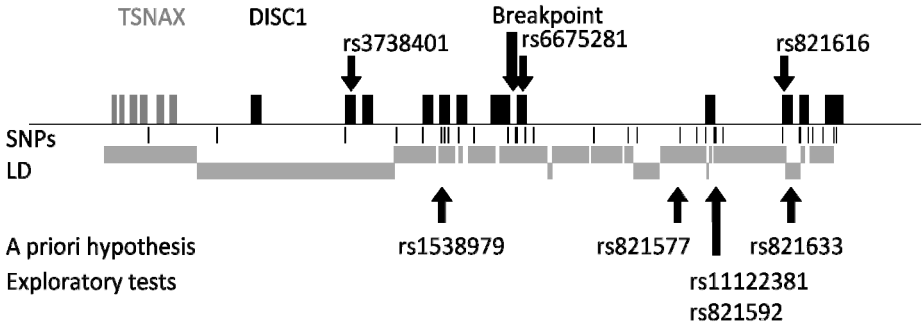
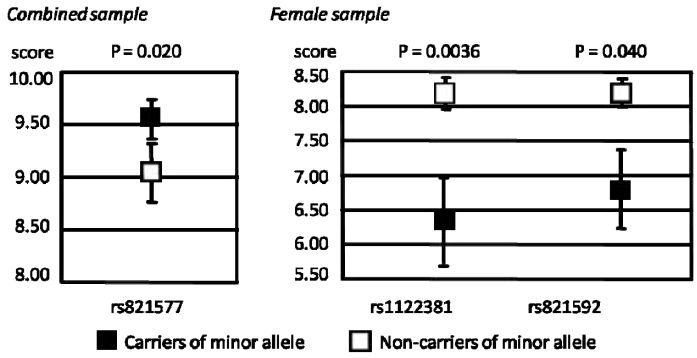
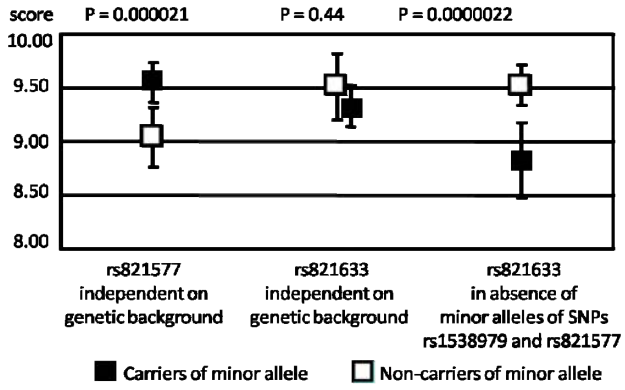


Figure 10. The schematic describes the study setting in Study III. As an a priori hypothesis three SNPs were studied for association with measures of psychosis proneness. In exploratory analyses, two additional SNPs displayed significant evidence for association.

a)



b)



c)

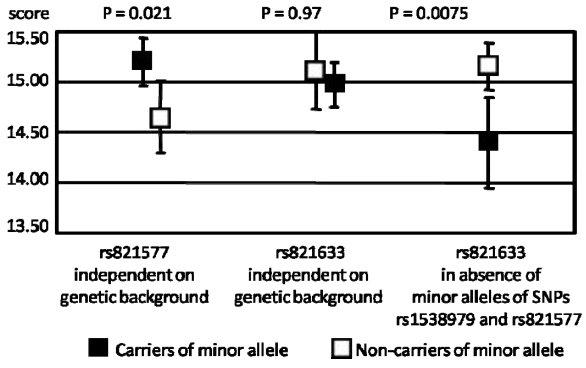


Figure 11. Mean values, 95% confidence intervals and corrected P-values for the significant markers on the Revised Social Anhedonia Scale in the exploratory analyses (a), and summary of the findings in the conditioned analyses for Revised Social Anhedonia Scale (b) and the Revised Physical Anhedonia Scale (c).

5.3.3 Conclusions

In the present study, we detected association between variants in *DISC1* and measures of anhedonia. A previous study by Hennah and colleagues had reported that three interdependent SNPs in *DISC1* contribute to the risk of schizophrenia and bipolar disorder. We were able to partially replicate this finding with the same variants in our study associating with measures of physical and social anhedonia. Additionally, we detected female-specific associations with two other SNPs in the exploratory analyses.

The exploratory association tests were corrected for multiple testing using the Bonferroni correction. This correction is obviously overly conservative because the studied phenotypes and markers are not independent of each other, association in the two sexes is not independent and dominant and recessive analyses are not independent. We preferred to err on the side of caution in our interpretation by using the overly conservative cutoff.

Our study was the first to report association between schizophrenia-related traits and *DISC1* at the population level. This notably adds to the previous evidence of *DISC1*'s important role in the etiology of schizophrenia. To estimate the gene's effects in the general population, unselected population cohorts have to be studied. Performing diagnostic and neuropsychological tests and subsequently looking for association between schizophrenia and the gene variants in large cohorts is difficult. First of all, given the prevalence of schizophrenia in the general population (1 %), unfeasibly large sample sizes are required. Performing diagnostic tests in such large cohort would require large resources. We avoided large costs by using validated pen-and-paper questionnaires. The ability of these scales to predict later onset psychotic disorders has been studied on a large scale. Both physical and social anhedonia are suggested to be efficient in predicting the onset of psychotic disorders later in life (Horan et al. 2008, Fonseca-Pedrero et al. 2009).

Naturally, not all individuals who score high on the scales develop psychotic disorders. However, the scales have no "normal" limit. Our findings suggest that *DISC1* might have an impact on how people psychologically react. Therefore, *DISC1* might be even more central to human psychological functioning than previously thought.

Our findings also support the idea of a complex interplay among genetic variants in the genetic background of schizophrenia. SNP rs821633 only displayed a significant effect when conditioned on two other SNPs.

In the exploratory analyses, we detected female-specific association with markers rs11122381 and rs821592. This region has been previously implicated in schizophrenia by Qu and colleagues who detected significant association between schizophrenia and a haplotype overlapping this region. Furthermore, these markers

are located in close proximity to the non-synonymous SNP rs821616. This SNP has associated with schizophrenia and related phenotypes in many previous studies (Qu et al. 2007, Kim et al. 2008, Thomson et al. 2005a, DeRosse et al. 2007) and displayed uncorrected evidence for association with the schizoidia measure in the present study.

DISC1 has been especially connected to the negative symptoms of schizophrenia. Variants in *DISC1* have previously displayed association with schizophrenia related learning and memory deficits also in the Finnish schizophrenia family (Hennah et al. 2005). Learning and memory deficits are also closely related to the negative aspects of schizophrenia. Our findings further strengthen the theory that *DISC1* is important in the negative aspects of schizophrenia.

5.4 *DISC1* conditioned genome-wide association study on psychosis proneness (Study IV)

The number of *DISC1* binding partners displaying evidence of involvement in the etiology of schizophrenia is increasing and 16 of the 27 known *DISC1* binding partners studied display evidence of involvement in the etiology of schizophrenia (Table 5, page 53). After finding strong evidence for association with anhedonia using the same *DISC1* variants that had previously associated with schizophrenia and bipolar disorder, we wanted to utilize these variants to recognize new *DISC1*-related susceptibility variants. We used a similar strategy as in Study I and conditioned a whole genome-wide SNP association analysis on the *DISC1* variants. We utilized the NFBC66 sample and the measures we previously detected association with: RSAS and RPAS. As in the study that identified *NDE1*, we hypothesized that with these genetically more homogeneous stratified samples we would be more likely to recognize variants with small effect sizes related to psychosis proneness that potentially interact with *DISC1*.

Association analyses between SNPs (a total number of SNPs: 331 307) and traits of social and physical anhedonia were performed in the whole sample using *DISC1* variants as covariates and separately in the three groups defined by individuals' *DISC1* variants. The number of individuals belonging to different *DISC1* variant groups were as follows: *DISC1* risk variant (N = 3049), *DISC1* protective variant (N = 962), and neutral *DISC1* variant (N = 505). In the analysis with *DISC1* as a covariate, a new variable based on the carried *DISC1* variants was created to be used as a covariate in the analyses.

No markers reached genome-wide significance at the level of P < 5E-7 using any of the models tested. To see if we observed lower p-values than expected by chance, P-

values were plotted in quantile-quantile plots (QQ-plots). No notable inflation from the level expected by chance was observed under any of the models tested (Figure 12). However, when the results were illustrated by plotting the –log P-values against all SNPs in order of their chromosomal positions (Manhattan plots) to observe any local clustering of low P-values, a number of suggestive clusters were observed (Figure 13). We followed up 18 of the most consistent clusters where at least three SNPs displayed P < 1E-4 by mapping the closest genes (UCSC Genome Browser, NCBI Build 36.1). SNPs with P-values < 1E-4 located in consistent clusters are presented in Table 13 together with information on their closest genes.

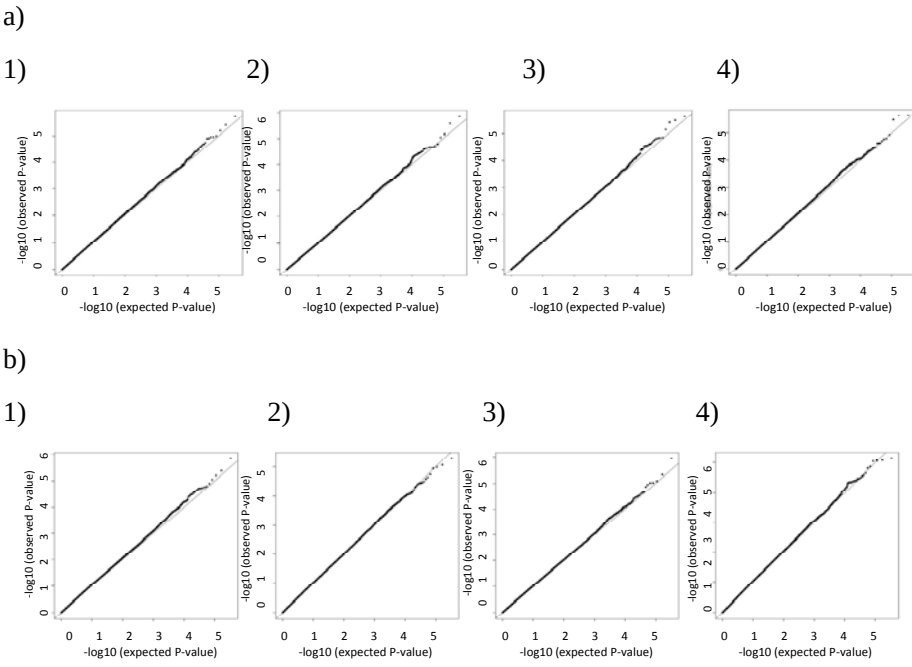


Figure 12. The schematics show the QQ plots for the observed –log10 P-values versus those expected by change for Revised Social Anhedonia Scale (a) and Revised Physical Anhedonia Scale (b) risk (1), protective (2), neutral (3) and covariated (4) models.

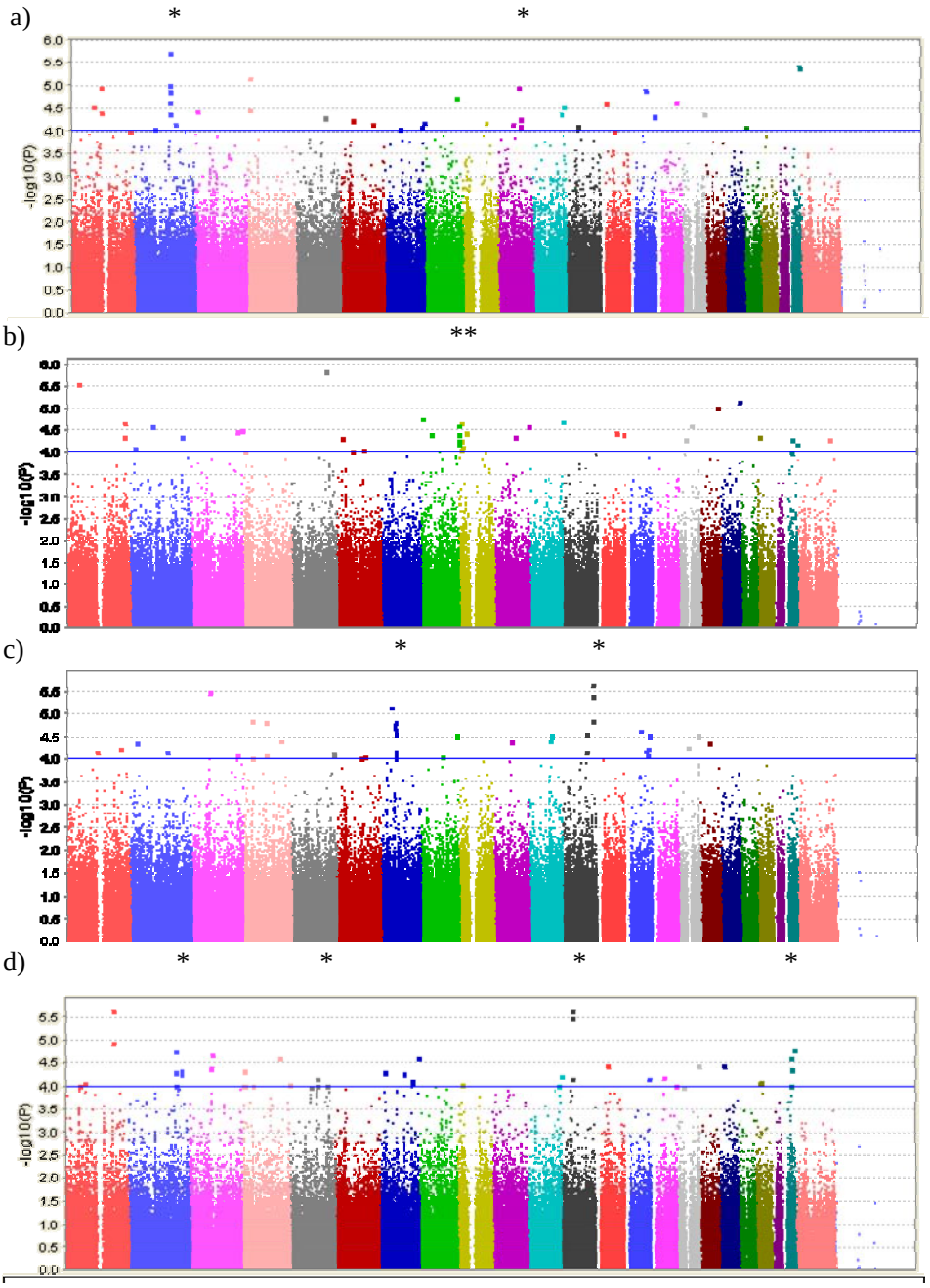


Figure 13. Manhattan plots for all the tested models: RPAS risk (a), RPAS protective (b), RPAS neutral (c), RPAS covariated (d), RSAS risk (e), RSAS protective (f), RSAS neutral (g), RSAS covariated (h). Blue line corresponds to P = 10 E-4. The clusters with ⩾ 3 SNPs with P < 10E-4 are marked with asterisk (*).

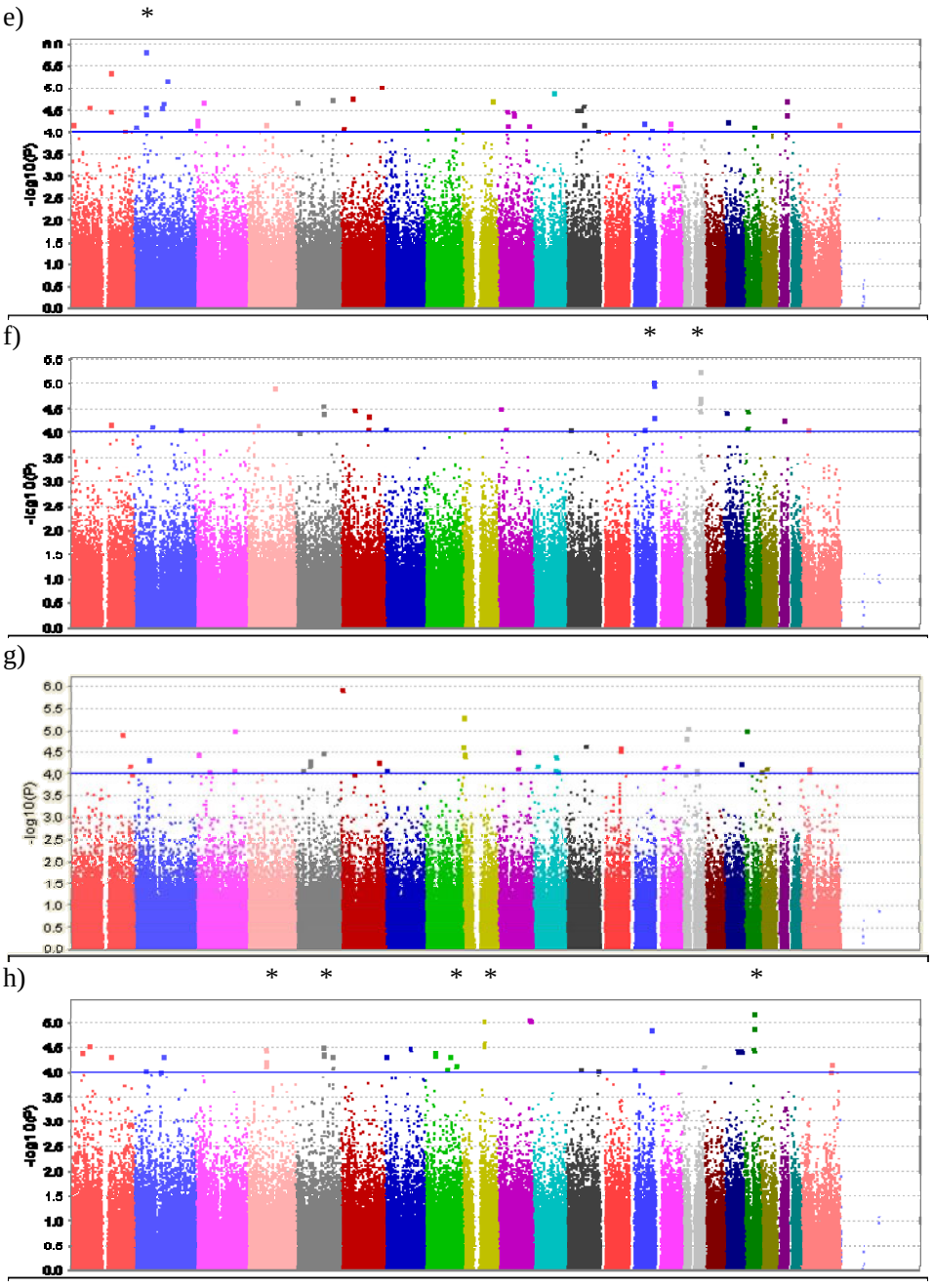

e)
f)
g)
h)
-log10(P)
Color key:
Chr1 Chr2 Chr3 Chr4 Chr5 Chr6 Chr7 Chr8 Chr9 Chr10 Chr11 Chr12
Chr13 Chr14 Chr15 Chr16 Chr17 Chr18 Chr19 Chr20 Chr21 Chr22 ChrX
ChrXY

Table 13. Summary of the SNPs displaying P-values <1E-4 located within clusters having consistently low P-values. For all the SNPs, the closest gene and the distance to the gene in base pairs are indicated.

Model	Chr	rs-number	Position (bp)	P-value	A	MAF	Closest gene	Dist (kb)
RSAS R	2	rs4953223	45370846	1.49E-06	G	0.385	UNQ6975	65
RSAS R	2	rs4953225	45374689	2.53E-05	G	0.340	UNQ6975	61
RSAS R	2	rs883413	45383099	3.67E-05	A	0.336	UNQ6975	52
RSAS P	14	rs2145631	95480113	8.97E-06	G	0.179	BC016484	35
RSAS P	14	rs999200	95488632	1.06E-05	C	0.181	BC016484	27
RSAS P	14	rs1998245	95498331	4.72E-05	A	0.238	BC016484	37
RSAS P	16	rs8062856	67640318	2.28E-05	A	0.453	TMCO7	0
RSAS P	16	rs11075704	67648719	5.30E-06	G	0.409	TMCO7	0
RSAS P	16	rs2232228	67701078	3.58E-05	G	0.365	HAS3	0
RSAS P	16	rs3785079	67702977	1.82E-05	A	0.331	HAS3	0
RSAS C	4	rs370655	75121998	5.61E-05	G	0.334	CXCL3	0
RSAS C	4	rs9131	75181913	3.43E-05	G	0.336	CXCL2	0
RSAS C	4	rs3806792	75184138	7.11E-05	A	0.332	CXCL2	0
RSAS C	5	rs10515379	107167562	3.13E-05	A	0.375	FBXL17	56
RSAS C	5	rs6888461	107174573	4.15E-05	A	0.386	FBXL17	49
RSAS C	5	rs7700475	107186057	4.31E-05	A	0.251	FBXL17	37
RSAS C	8	rs7833633	35529967	4.12E-05	G	0.069	UNCD5	0
RSAS C	8	rs6980691	35531940	4.23E-05	C	0.069	UNCD5	0
RSAS C	8	rs983271	35534194	4.33E-05	G	0.067	UNCD5	0
RSAS C	9	rs4554557	84714350	2.41E-05	A	0.178	RASEF	73
RSAS C	9	rs12340476	84730049	8.71E-06	A	0.173	RASEF	57
RSAS C	9	rs17085898	84757459	2.92E-05	A	0.156	RASEF	30
RSAS C	19	rs8110509		6.35E-06	A	0.243	ZNF536	0
RSAS C	19	rs919803	35678944	3.52E-05	G	0.296	ZNF536	0
RSAS C	19	rs3786791	35718715	1.27E-05	A	0.271	ZNF536	0
RPAS R	2	rs2305165	136126044	2.14E-05	C	0.092	R3HMD1	0
RPAS R	2	rs4954633	136263105	1.84E-06	A	0.102	LCT	0
RPAS R	2	rs2874874	136294664	1.01E-05	C	0.111	LCT	0
RPAS R	2	rs3754690	136307028	1.31E-05	A	0.112	LCT	0
RPAS R	2	rs309126	136360370	4.02E-05	G	0.129	MCM6	10
RPAS R	10	rs10509494	86142888	5.36E-05	A	0.077	KIAA1128	0
RPAS R	10	rs1763976	86247427	7.91E-05	A	0.077	KIAA1128	0
RPAS R	10	rs1777112	86251786	7.92E-05	A	0.078	KIAA1128	0
RPAS R	10	rs1188774	86297467	7.73E-05	A	0.073	KIAA1128	29
RPAS P	8	rs199215	140800438	6.14E-05	A	0.160	TRAPPC9	11
RPAS P	8	rs199231	140811036	2.49E-05	A	0.158	TRAPPC9	1
RPAS P	8	rs898663	140819354	2.49E-05	C	0.156	TRAPPC9	0
RPAS P	8	rs11773968	140840193	5.38E-05	A	0.068	TRAPPC9	0
RPAS P	8	rs11781035	140840343	3.69E-05	A	0.150	TRAPPC9	0
RPAS P	9	rs2997564	7444698	8.79E-05	C	0.334	C9orf123	34

Model	Chr	rs-number	Position (bp)	P-value	A	MAF	Closest gene	Dist (kb)
RPAS P	9	rs2997570	7450556	2.19E-05	A	0.351	C9orf123	34
RPAS P	9	rs2765970	7452518	2.19E-05	C	0.351	C9orf123	33
RPAS P	9	rs1926370	7461079	5.36E-05	G	0.463	C9orf123	33
RPAS N	7	rs11766051	55806039	2.59E-05	A	0.133	PSPHL	2
RPAS N	7	rs10243293	55809594	2.73E-05	A	0.136	PSPHL	1
RPAS N	7	rs6966061	55816284	2.44E-05	G	0.133	PSPHL	8
RPAS N	7	rs9642404	55824293	1.46E-05	A	0.157	SEPT14	4
RPAS N	7	rs1113765	55856828	9.59E-05	A	0.256	SEPT14	0
RPAS N	7	rs10230845	55873390	2.11E-05	A	0.139	SEPT14	0
RPAS N	7	rs11761352	55918441	6.33E-05	A	0.152	MRPS17	4
RPAS N	7	rs10271662	55930401	8.36E-05	C	0.164	MRPS17	0
RPAS N	12	rs11610671	114895945	3.86E-06	A	0.231	MED13L	0
RPAS N	12	rs10507268	115024610	1.39E-05	A	0.237	MED13L	0
RPAS N	12	rs11615689	115184058	2.24E-06	G	0.234	MED13L	0
RPAS C	2	rs4894030	179169467	9.40E-05	C	0.176	TTN	0
RPAS C	2	rs2046778	179380792	1.67E-05	G	0.170	TTN	0
RPAS C	2	rs1873164	179461794	4.82E-05	G	0.253	CCDC141	0
RPAS C	5	rs319131	103353873	6.45E-05	A	0.418	NUDT12	427
RPAS C	5	rs6892281	103547741	9.52E-05	G	0.394	NUDT12	621
RPAS C	5	rs3931126	103561783	9.54E-05	A	0.394	NUDT12	635
RPAS C	12	rs4768236	39042739	2.29E-06	C	0.263	LRRK2	0
RPAS C	12	rs3761863	39044919	3.34E-06	A	0.260	LRRK2	0
RPAS C	12	rs9668854	39105985	6.46E-05	A	0.094	LRRK2	57
RPAS C	22	rs929014	31488023	4.24E-05	A	0.210	SYN3	0
RPAS C	22	rs2899194	31497851	2.47E-05	A	0.242	SYN3	0
RPAS C	22	rs130559	31518536	9.28E-05	A	0.231	SYN3	0

Abbreviations: RSAS: Revised Social Anhedonia Scale, RPAS: Revised Physical Anhedonia Scale, Chr: chromosome, A: minor allele, MAF: minor allele frequency, dist kp: distance to the closest gene in kilo base.

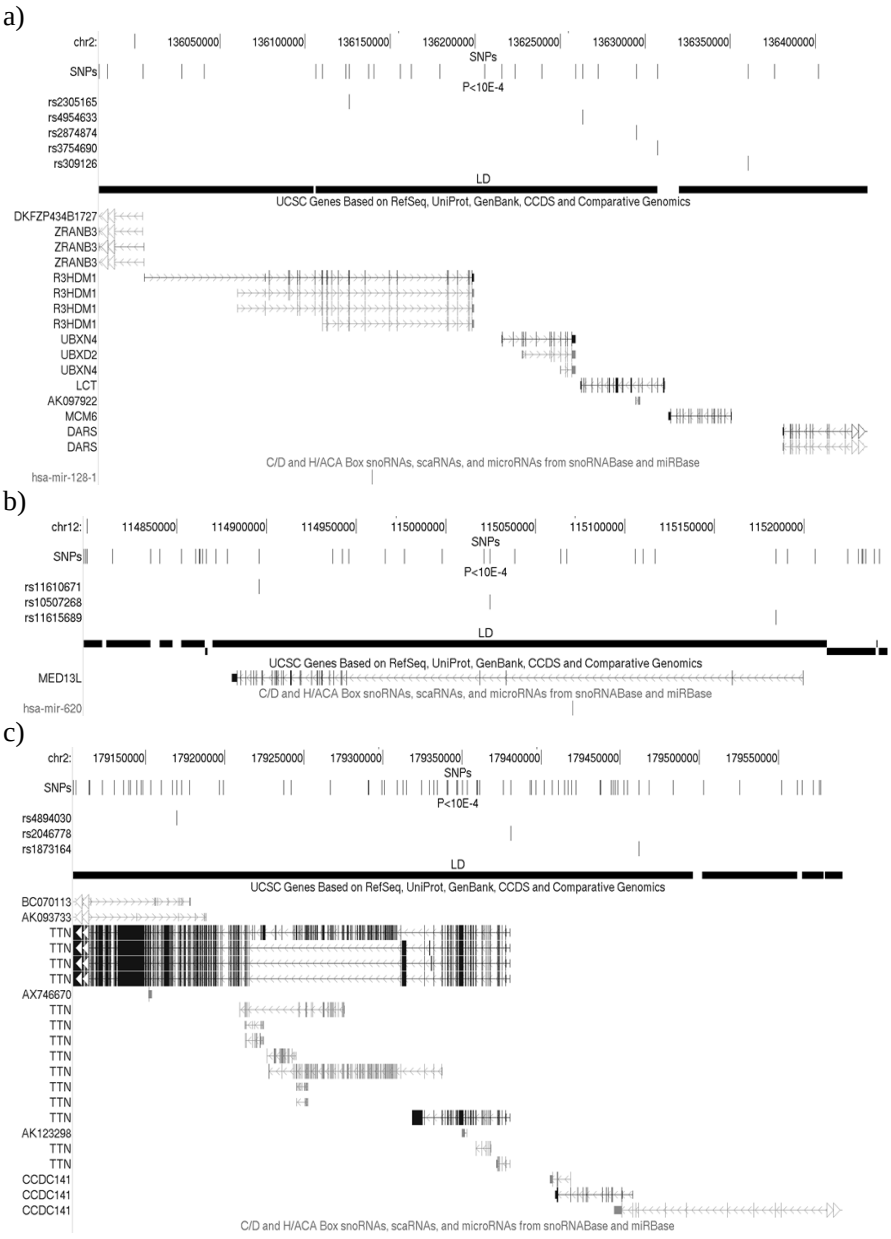


Figure 14. The schematics show the regions with suggestive evidence for association on 2q31.2 (a), 2q21.3 (b) and 12q24.21 (c). The figures are based on UCSC genome browser information as of March 2006 (NCBI36/hg18). All the SNPs genotyped on the regions are indicated with vertical lines. For the SNPs with P < 1E-4 the rs-numbers are also provided. Genes (UCSC Genes) and miRNAs located within the regions are displayed.

To estimate the relevance of the genes displaying suggestive evidence for association in the *DISC1* related pathways, we utilized the Ingenuity Pathway Analysis tools. Both direct and indirect connections between *DISC1* and other DISC1 pathway genes as well as the genes displayed in Table 13 were studied. SNP rs1873164 on chromosome 2 that displayed evidence for association with RPAS (P = 4.82E-05, MAF = 0.253) in the analysis with *DISC1* as a covariate is located within *CCDC141* gene, which was shown to interact with *DISC1* directly (Figure 14c). Even though other genes could not be directly linked to DISC1 pathways through biological interactions, it has been previously shown that expression levels of *LCT* (rs4954633, P = 1.84E-06, MAF = 0.102) are influenced by variants in *DISC1* (Figure 14a) (Hennah et al. 2009a).

Recently many non-coding genes have been implicated in the etiology of schizophrenia and other major mental illnesses (Kocerha et al. 2009). Therefore, we wanted to see if non-coding genes are also implicated in the present study. We searched the UCSC database for non-coding micro RNA (miRNA), small nuclear RNA (sno-RNA) and small interfering (siRNA) genes located at the cluster regions. MiRNAs were located within two cluster regions. On chromosome 2q21.3 in close proximity to *LCT, MIR128-1* is located (Figure 13b). In the 12q24.21 region, several markers displayed suggestive evidence for association with physical anhedonia only when the carriers of the neutral *DISC1* variant were included in the analyses. Within this region, SNP rs11615689 displayed the strongest evidence for association (P = 2.24E-06; MAF = 0.23). At this locus, *MIR620* is located (Figure 14b).

We studied the miRNAs' predicted target sites. The predicted target sites were recognized using a publicly available database miRBase (Griffiths-Jones et al. 2008, Griffiths-Jones et al. 2006, Griffiths-Jones 2004). The miRNA MIR620 regulates the expression levels of 778 genes. In the Ingenuity pathway analysis, these genes were significantly enriched in pathways related to bipolar disorder (67 molecules; P = 6.69E-06). Physiologically the second most enriched category is nervous system development (39 molecules). These genes included five genes (*GABRA1, GRIK1, FOXP2, NUMBL* and *PCP4*) that have been previously implicated in the etiology of schizophrenia through expression changes. MIR128-1 had 755 predicted target sites, but they did not display enrichment in pathways related to central nervous system or mental illnesses.

5.4.1 Conclusions

We performed a whole genome-wide scan conditioned on the *DISC1* variants that associate with schizophrenia, bipolar disorder and psychosis proneness measures of physical and social anhedonia. The aim was to recognize new variants dependent on *DISC1* that play roles in the etiologies of major mental illness. Even though no

significant association was detected, we detected some evidence for association within loci that have biological relevance in the etiology of schizophrenia.

One of the most significantly associated genes was the *LCT* gene. It is suggested to be an indicator of population stratification due to large variation in allelic frequencies across different populations (Campbell et al. 2005, Epstein et al. 2007). In a previous study by Jakkula and colleagues (Jakkula et al. 2008), a substructure analysis using Eigensoft analysis methods (Patterson et al. 2006) was performed on the Finnish population. The analysis included a subset of SNPs used in the present study and the same population cohort was used as a subpopulation. For the variants recognized in the present study, the first two eigenvectors did not display any evidence for population stratification within our sample.

Intriguingly, *LCT* is one of the target sites of miRNA MIR620 that is located in a genomic region displaying evidence for association under the *DISC1*-neutral model. Furthermore, in a previous report by Hennah and colleagues the expression level of *LCT* was influenced by *DISC1* variants.

Numerous miRNAs have been implicated in the etiology of schizophrenia and other mental illnesses. These include *miR-9* and *miR-124* involved in neuronal development, and miRNAs *miR-134* and *miR-138* involved in neuronal plasticity (Christensen et al. 2009). In our analysis, *MIR620* displayed evidence for involvement in the etiology of mental illnesses. Predicted target sites of MIR620 were enriched in central nervous system development pathways genes (Ingenuity Pathway Analysis). Five of the genes have been previously implicated in the etiology of schizophrenia because of their expression changes (Petryshen et al. 2005, Choi et al. 2009, Passos Gregorio et al. 2006, Fatemi et al. 2008b, Byne et al. 2009). Furthermore, the predicted target sites of MIR620 were also significantly enriched in the bipolar disorder pathways. Patients with bipolar disorder display elevated RPAS scores while relatives of affected individuals and healthy controls have similar scores (Etain et al. 2007). Therefore, RPAS is not considered a bipolar disorder endophenotype (Etain et al. 2007). However, a connection between RPAS and depression, a central symptom of bipolar disorder, has been suggested (Leventhal et al. 2006). Our findings might thus suggest that *MIR620* has a role in the brain and in the etiology of mental illnesses by regulating numerous genes contributing to the onset of mental disorders

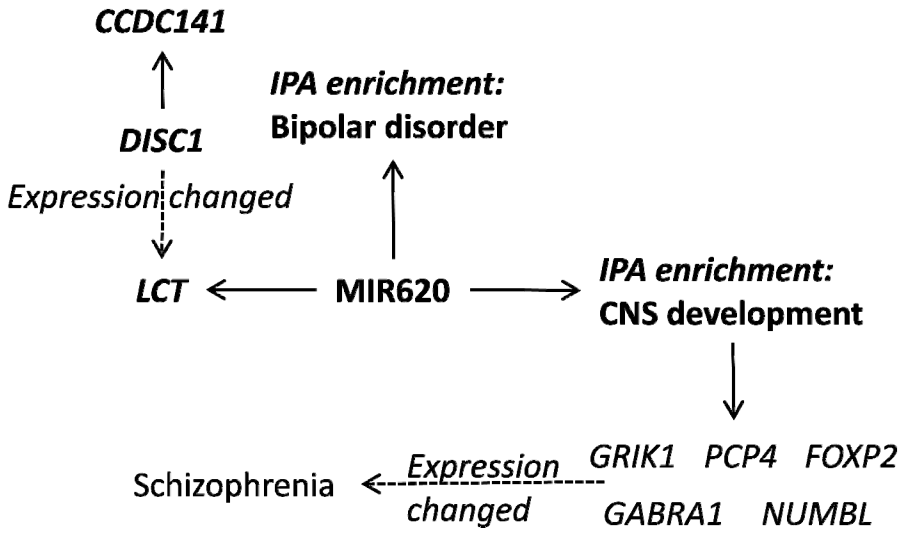


Figure 15. The schematic describes the most interesting findings and how they are related to DISC1 and biological functions/pathways in the background of schizophrenia.

6 Concluding remarks and future prospects

We have studied the common variance in the genetic background of schizophrenia in a pathway-driven manner. We have studied the genes comprising one of the most promising candidate networks for schizophrenia: the DISC1 pathway.

We utilized a novel conditioned genome-wide linkage approach to recognize new variants contributing to the onset of schizophrenia. By conditioning the study on a *DISC1* risk variant HEP3 that was recognized in the Finnish families ascertained for schizophrenia, we detected significant linkage on a locus where a DISC1 binding partner *NDE1* is located. Variants in *NDE1* displayed significant independent association with schizophrenia within the same sample. We continued by studying other genes in *DISC1* pathway using a candidate gene approach. We selected the genes for study by evidence of their biological interaction between the gene products. We found strong evidence for the involvement of *NDEL1*, *PDE4B* and *PDE4D* in addition to *NDE1* and *DISC1* in the etiology of schizophrenia.

We have also found important additional evidence for *DISC1* itself. Variants in this gene associated significantly with anhedonia in the general healthy population. The associating variants were the same that previously displayed an allelic interplay that associated with both schizophrenia and bipolar disorder. This finding was important for at least two reasons. First, our replication strengthens the idea of a complex allelic interplay between genetic variants in the genetic background of schizophrenia. Secondly, with this finding, we were able to for the first time show an impact of *DISC1* on a psychosis related trait in the Finnish general population. This way, *DISC1* gene might be relevant, not only in terms of mental illness, but also in more general sense in psychological functioning.

Finally, through *DISC1*-conditioned genome-wide association we recognized additional potentially interesting loci involved in the etiology of schizophrenia. *LCT*, *CCDC141*, *MIR620* and *MIR128-1* displayed evidence for *DISC1*-conditioned association with physical anhedonia. They provide promising new candidate molecules for further study in the genetic background of schizophrenia.

In the present study, we have utilized intermediate phenotypes for schizophrenia in addition to studying genetic association with the schizophrenia diagnosis. These phenotypes included learning and memory related endophenotypes of schizophrenia and intermediate psychosis proneness measures. Endophenotypes are thought to result from a more simple genetic architecture compared to a clinical phenotype, and thus they facilitate the recognition of genetic susceptibility variants.

Endophenotypes might be the manifestation of the cellular and molecular changes (Benes 2007). However, the overlaps between endophenotypes and clinical phenotypes are significant in schizophrenia, other psychotic disorders and bipolar disorder (Benes 2007). Similarities in the clinical phenotype suggests that the respective endophenotypes may also show areas of overlap with respect to the circuitry involved and the nature of the cellular and molecular changes present (Benes 2007).

The genetic etiology of schizophrenia is heterogeneous. Numerous different variants within the same genes have contributed to the risk in different samples. Therefore, it seems that numerous different variants within a number of genes can result in a similar phenotype. This might explain why meta-analyses have often failed to display congruent evidence for even the "best" candidate genes. This is the case for *DISC1* and the DISC1 binding partners. When studying such a heterogeneous disorder, it is worthwhile to take advantage of an isolated population, such as the population of Finland. In such populations, the amount of genetic variation in the background of a certain disorder is assumed to be smaller than in a more mixed population, and thus the recognition of susceptibility variants and genes is easier.

In today's large-scale genome-wide studies, the amount of genetic data available for analysis has remarkably increased. The trend will continue into the future as genome-wide sequencing becomes a more easily accessible tool for genetic studies. This will create challenges for the analysis and interpretation of genetic data. Along with statistical significance, the pathway approaches we have utilized in the present study might help with the processing of massive amounts of data. Furthermore, the studies in the future will likely benefit from combining existing biological evidence with genetic data, especially when interpreting results and their significance.

The ever increasing understanding of human genetic variation and non-coding genes, such as miRNAs and siRNAs, and their involvement in human diseases will provide new targets for genetic studies. Numerous miRNAs have already been implicated in psychiatric and neurodevelopmental disorders, including the miRNAs for which we detected association. However, the function of a large proportion of the DNA sequence is still unknown (so-called junk-DNA). New, advanced methods could eventually reveal the function of junk-DNA and provide new insights into the genetic mechanisms behind disorders.

When the present study was initiated in 2005, only the genes *DISC1*, *FEZ* and *PDE4B* out of the large group of DISC1 binding partners had been implicated in the etiology of schizophrenia. Since then, the number of *DISC1*-related susceptibility genes implicated in the etiology of schizophrenia has been growing quickly. Intriguingly, in addition to the recognition of new schizophrenia candidate genes in these pathways, many of the "old", strong candidate genes have shown to be closely related to DISC1 pathways. These include *NRG1*, *RELN*, *AKT1* and *COMT*

(Nicodemus et al. 2007). New, more efficient study techniques have enabled and will enable much more efficient genotyping of the candidate genes in the future. This will greatly facilitate the recognition of new susceptibility genes.

The increasing understanding of biological interactions among genes and gene products will also facilitate genetic studies. It has already been shown that genetic interplay in the DISC1 pathway within genes (Hennah et al. 2009b) and between different genes (epistasis) (Burdick et al. 2008, Nicodemus et al. 2010) contribute to the risk of mental illnesses. As the understanding of biological interplay between genes and gene products increases, studying these interactions with respect to phenotypes becomes easier. It has been argued that studying single, independent SNPs for complex disorders is not optimal since the effect of genetic variation is dependent on the genetic environment. In the future, genetic studies will most likely study genetic networks rather than single genes.

In addition to genetic interplay, gene x environment interactions (G x E) contribute significantly to mental illnesses. Thus, in the future, the studies of schizophrenia will emphasize complex interplay between genetic and environmental factors. Numerous significant gene x environment interactions have already been reported. These include interactions between obstetric complications and *AKT1*, *GRM3*, *BDNF1* and *DTNBP1* and cannabis use and *COMT* (Roth et al. 2009, Nicodemus et al. 2008, Caspi et al. 2005). *DISC1* and the DISC1 pathway have not yet been explored for the gene x environment interactions, thus it would be of interest to study the genes and the variants we have recognized for interactions with environmental factors. Due to the involvement of the *DISC1* in neuronal development, fetal infections or obstetric complications might provide phenotypes for studying such interactions.

After our reports on the predisposing variants in *NDE1*, *NDEL1*, *PDE4B* and *PDE4D*, these variants have been shown to significantly affect expression levels of numerous genes significantly enriched in neurodevelopmental, synaptogenic and sensory perception gene ontology (GO) pathways. Intriguingly, several genes coding for proteins that are psychiatric drugs targets displayed altered expression rates dependent on variants in NDE1 and PDE4B (Hennah et al. 2009a). The PDE4s themselves are targets for rolipram, a medicine having antidepressant, antipsychotic and cognitive enhancement effects (Rose et al. 2005). Furthermore, AKT1 interacting with DISC1 through GSK3β is a target for mood-stabilizer lithium (Ming et al. 2009). This suggests that the DISC1 pathway might offer targets for further drug development (Hennah et al. 2009a). Based on these findings, DISC1 pathway genes might even contribute to an individual's response to the current psychiatric medication.

The ultimate goal in genetic studies is naturally the recognition of functional variants behind the diseases. This would eventually enable better understanding about the studied disorders and enable the design of better or new treatments. The

present study does not provide any conclusive or definitive findings on *DISC1* or the DISC1 pathway. However, our findings strongly support the role of the DISC1 pathway in the etiology of schizophrenia and human mental functioning. In case of multifactorial, complex disorders it unlikely to recognize actual disease causing variants trough genetic association studies. Rather, the recognition of true variants requires more accurate genetic analyses such as sequencing. The growing evidence for especially *DISC1* and also *PDE4B* would suggest that these genes might be good targets for more precise genetic analyses in the future (i.e. sequencing). The recognition of actual functional variants for a number of key molecules on the DISC1 pathways might facilitate recognition of remaining variants as well.

By further understanding how DISC1 pathway genes possibly contribute to treatment outcomes and drug responses, it might be possible to target future medication more precisely. In schizophrenia, this would be a big step towards better treatment. Currently more that 20 % of schizophrenia patients do not initially respond to treatment with drug therapy. In addition, many patients discontinue their medication due to their side effects (Gerretsen et al. 2009). With additional information on the genetic profiles affecting these factors, it might be possible to achieve better treatment outcomes. Naturally, to be able to design treatment based on patients' genetic profiles, additional genetic tests have to be created as well.

Phenotypic assessment poses certain challenges for the studies of schizophrenia and other psychiatric disorders in comparison with other complex disorders like celiac disease and dyslipidemias. Schizophrenia is phenotypically very heterogeneous. The diagnosis is clinically based on a collection of various symptoms. Furthermore, schizophrenia symptoms are not at all specific for schizophrenia. They include many of the symptoms typical of other psychiatric disorders as well, and thus there is significant overlap in the phenotypic appearance. Most of the psychiatric disorders also seem to share a significant part of their genetic liability. It is often questioned if schizophrenia is an independent disorder or rather a group of disorders with highly similar phenotypes. It has been suggested that psychiatric disorders could be managed as a continuum of psychiatric symptom dimensions rather than strictly categorized independent diagnoses (van Os et al. 2009). Additional understanding of the genetic and patophysiological components underlying the symptoms of schizophrenia and other psychiatric disorders might eventually help in diagnostic classification and assessment (van Os et al. 2009, Korth 2009). Furthermore, it was recently suggested that some of the candidate genes for schizophrenia such as DRD2, APOE and NRG1 might provide blood biomarkers for psychosis (Kurian et al. 2009). This might, in the future, enable more precise diagnosing and treatment of psychotic disorders. The future will show whether the genes of the DISC1 pathway can be utilized this way.

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