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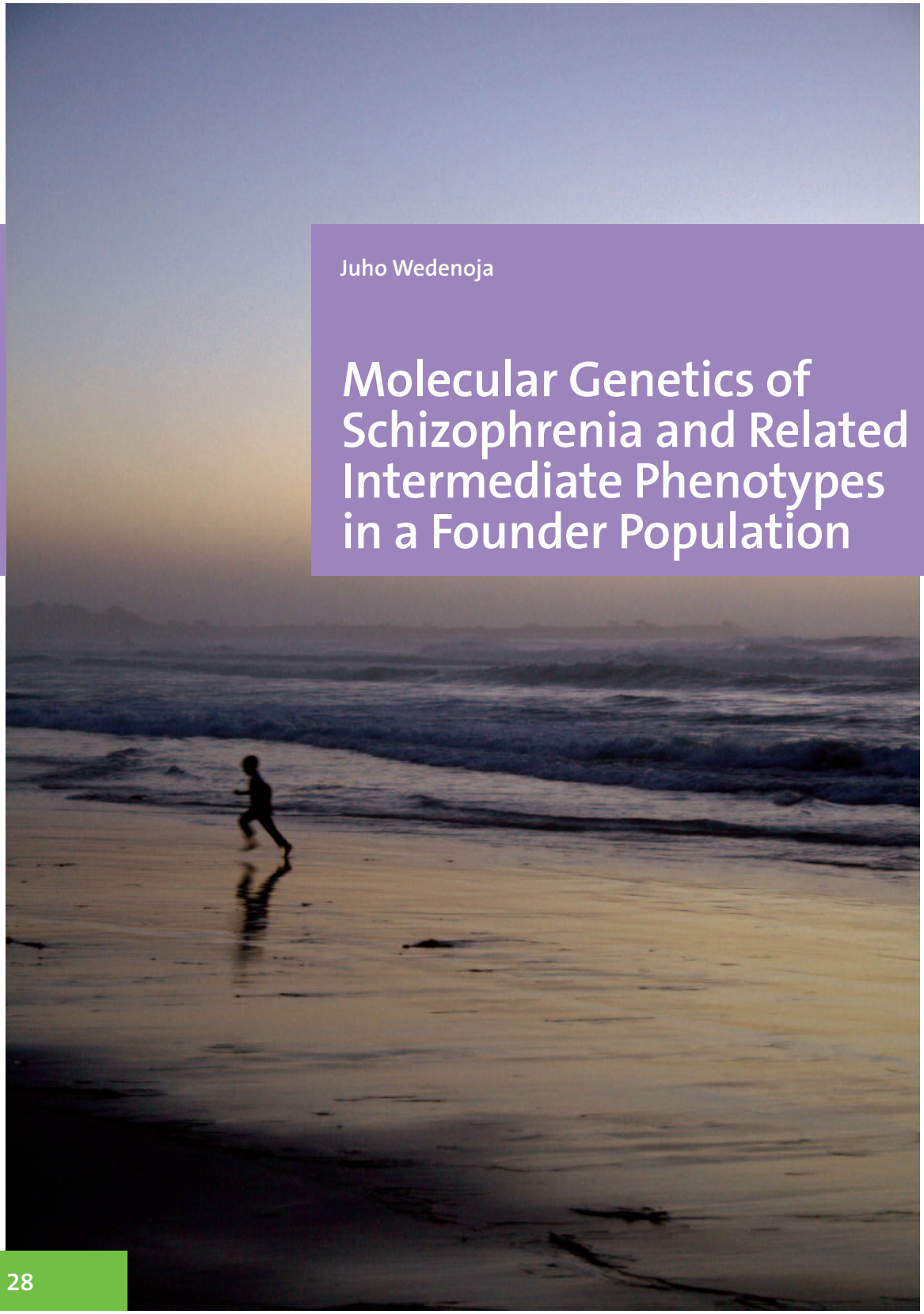

RESEARCH

Juho Wedenoja

Molecular Genetics of Schizophrenia and Related Intermediate Phenotypes in a Founder Population

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Molecular Genetics of Schizophrenia and Related Intermediate Phenotypes in a Founder Population

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine, University of Helsinki, for public examination in the Small Hall, University of Helsinki Main Building, on 5 February 2010, at 12 noon.

National Institute for Health and Welfare, Helsinki, Finland
and
Institute for Molecular Medicine Finland FIMM, Helsinki, Finland
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RESEARCH 28
Helsinki 2010

Helsinki University Biomedical Dissertations No 131

ISSN 1457-8433

Publications of the National Institute for Health and Welfare Research 28

ISBN 978-952-245-219-1 (print)
ISSN 1798-0054 (print)
ISBN 978-952-245-220-7 (pdf)
ISSN 1798-0062 (pdf)

Cover picture: Satu Wedenoja (Monterey, CA, USA on 7 November 2007)

http://www.thl.fi/julkaisut
http://ethesis.helsinki.fi/

Helsinki University Print
Helsinki, Finland 2010

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"Any sufficiently advanced technology is indistinguishable from magic."

- Arthur C. Clarke -

To Satu

Juho Wedenoja. Molecular Genetics of Schizophrenia and Related Intermediate Phenotypes in a Founder Population.
National Institute for Health and Welfare, Research 28, 101 pages
Helsinki, Finland 2010
ISBN 978-952-245-219-1 (print); ISBN 978-952-245-220-7 (pdf)

Abstract

Schizophrenia is a severe mental disorder characterized by positive and negative symptoms, such as psychosis and anhedonia, as well as cognitive deficits. Schizophrenia affects about 0.5 to 1% of population worldwide, with equal prevalence between the genders and different residential environments. That schizophrenia constitutes a significant burden for both the affected individuals and for the whole societies has encouraged its active research over the past decades. At present, schizophrenia is considered a complex disorder of neurodevelopmental origin with both genetic and environmental factors contributing to its onset. Despite the established strong genetic component, any major genetic determinant has remained unidentified.

The current hypothesis for schizophrenia liability comprises multiple genetic variants with small individual effects in conjunction with environmental factors. These combinations of genetic variants may differ substantially between the affected individuals, especially between the families not sharing the same ancestry. On the other hand, the same variants may cause different disorders depending on their combinations. Although a number of candidate genes for schizophrenia have been highlighted, only very few schizophrenia patients are likely to share identical genetic liability.

Close relatives of schizophrenia patients have an elevated risk for both schizophrenia and so-called schizophrenia spectrum disorders. This favors the use of family samples in genetic studies of schizophrenia. Furthermore, samples collected from isolated populations with increased genetic homogeneity may assist in identification of predisposing variants. This study is based on the nation-wide schizophrenia family sample of the National Institute for Health and Welfare. This wide series, collected from the relatively isolated Finnish population with limited genetic variation, represents one of the largest and most well-characterized familial series in the world.

In the first part of this study, we investigated the roles of the *Dystrobrevin binding protein 1* (*DTNBP1*), *Neuregulin 1* (*NRG1*), and *V-akt murine thymoma viral oncogene homolog 1* (*AKT1*) genes in the background of schizophrenia in Finland. Although these genes are associated with schizophrenia liability in several populations, any significant association with clinical diagnostic information of schizophrenia remained absent in our sample of 441 schizophrenia families. Therefore, our study provides no support for any major role of these genes behind schizophrenia in the Finnish population.

In the second part of this study, we first replicated schizophrenia linkage on the long arm of chromosome 7 in 352 schizophrenia families. In the following association analysis, we utilized additional clinical disorder features and intermediate phenotypes—endophenotypes—in addition to diagnostic information from altogether 290 neuropsychologically assessed schizophrenia families. An intragenic short tandem repeat (STR) allele of the regional *Reelin* (*RELN*) gene, supposed to play a role in the

background of several neurodevelopmental disorders, showed significant association with poorer cognitive functioning and more severe schizophrenia symptoms. Importantly, the effect of the risk allele on cognition was replicated in an independent subsample, and interestingly, its effect was stronger among the individuals affected with psychosis in the whole sample. Although any significant association with the clinical diagnoses remained absent, this risk allele was significantly more prevalent among the individuals affected with schizophrenia spectrum disorders. Our results support the involvement of *RELN* in schizophrenia liability, and especially, its role as a genetic modifier of the disorder features.

The wide spectrum of additional diagnostic information available in altogether 293 schizophrenia families was further utilized in the last part of this study. We have previously identified linkage of schizophrenia and its cognitive endophenotypes on the long arms of chromosomes 2, 4, and 5. Here, we selected altogether 104 functionally relevant candidate genes from the linked regions, and performed association analysis of clinical diagnostic categories, clinical disorder features, and several endophenotypic traits representing the central cognitive functions impaired in schizophrenia. Our approach allowed identification of several promising associations, of which especially interesting are the *Verb-a erythroblastic leukemia viral oncogene homolog 4* (*ERBB4*) gene, showing association with the severity of schizophrenia symptoms and impairments in traits related to verbal abilities, and the *Glutamate receptor, ionotropic, AMPA 1* (*GRIA1*) gene, showing association with the severity of schizophrenia symptoms.

This study supports the view that due to the heterogeneity of the disorder, sole clinical diagnostic information may be insufficient for detection of all predisposing variants for schizophrenia, and supposedly, for psychiatric disorders overall. Our results extend the previous evidence that the genetic risk for schizophrenia is at least partially mediated via the effects of the candidate genes and their combinations on relevant brain systems, resulting in alterations in different disorder domains, such as the cognitive deficits. Therefore, these results encourage the use of detailed disorder-related features and intermediate factors to extract maximal information from the study material in the search for specific risk variants.

Keywords: schizophrenia, psychiatry, linkage analysis, association analysis, founder population, endophenotype

Juho Wedenoja. Molecular Genetics of Schizophrenia and Related Intermediate Phenotypes in a Founder Population.
Terveyden ja hyvinvoinnin laitos, Tutkimus 28, 101 sivua
Helsinki 2010
ISBN 978-952-245-219-1 (painettu); ISBN 978-952-245-220-7 (pdf)

Tiivistelmä

Skitsofrenia on vakava mielenterveyden häiriö, jonka keskeisiä piirteitä ovat positiiviset ja negatiiviset oireet, kuten aistiharhat ja tunteiden latistuminen, sekä heikentynyt kognitiivinen suoriutuminen. Skitsofrenia alkaa yleensä nuorella aikuisiällä ja siihen sairastuu elinaikanaan arviolta 0,5-1,0 % maailman väestöstä. Skitsofrenian esiintyvyydessä ei ole merkittävää eroa eri sukupuolten tai maantieteellisten alueiden välillä. Sairauden aiheuttama merkittävä sekä inhimillinen että yhteiskunnallinen taakka ovat kannustaneet taudin aktiiviseen tutkimukseen viimeisten vuosikymmenien aikana. Nykykäsityksen mukaan skitsofrenia on keskushermoston kehityshäiriöstä johtuva monitekijäinen sairaus, jonka puhkeamiseen vaikuttavat sekä perimä että ympäristötekijät. Vaikka skitsofreniaan liittyy vahva geneettinen alttius, yksittäisiä korkean riskin alttiusgeenejä ei ole toistaiseksi tunnistettu.

Skitsofrenian taustalla oleva perinnöllinen alttius johtuu todennäköisesti useiden eri geenien vaikutuksesta. Vaikka yksittäisiin alttiusgeeneihin liittyvä sairastumisriski on luultavasti matala, useiden alttiusgeenien skitsofrenialle altistavat muodot voivat yhdessä vaikuttaa merkittävästi yksilön sairastumisriskiin. Siten erilaiset geenimuodot ja niiden yhdistelmät voivat vaihdella huomattavastikin paitsi sairastuneiden henkilöiden myös eri väestöjen välillä. Toisaalta samojen geenimuotojen eri yhdistelmät saattavat altistaa skitsofrenian lisäksi myös muille vakaville mielenterveyden häiriöille. Vaikka lukuisia skitsofrenialle altistavia geenimuotoja on tunnistettu, todennäköisesti vain harvoilla sairastuneilla on taudin taustalla samanlainen altistavien geenimuotojen yhdistelmä.

Skitsofreniaan sairastuneiden henkilöiden lähisukulaisilla on lisääntynyt riski sairastua sekä skitsofreniaan että muihin niin kutsuttuihin skitsofreniakirjon sairauksiin. Tämän vuoksi perheaineistojen käyttö voi auttaa skitsofrenialle altistavien geenimuotojen tunnistamisessa. Väestötasolla geneettinen samankaltaisuus vaikuttaa merkittävästi myös sairauksiin liittyvien alttiusgeenien kirjoon. Siten asutushistoriansa vuoksi eristäytyneiden väestöryhmien, kuten suomalaisten, geneettisen vaihtelun vähäisyys voi auttaa alttiusgeenien tunnistamisessa. Tämä tutkimus perustuu Terveyden ja hyvinvoinnin laitoksen keräämään suomalaiseen skitsofreniaperheaineistoon, joka on paitsi laajuudeltaan myös suomalaisen väestön geneettiset erityispiirteet huomioiden merkittävä koko maailmankin mittakaavassa.

Tutkimuksen ensimmäisessä osatyössä kartoitettiin *Dystrobrevin binding protein 1* (*DTNBP1*), *Neuregulin 1* (*NRG1*) ja *V-akt murine thymoma viral oncogene homolog 1* (*AKT1*) -geenien merkitystä skitsofrenian taustalla. Vaikka näiden geenien eri muodot on yhdistetty lisääntyneeseen skitsofreniariskiin useissa eri väestöryhmissä, tutkimuksessamme ei havattu merkittävää yhteyttä sairauden ja kyseisten geenien välillä 441 perheen aineistossamme. Siten tulosten perusteella vaikuttaa todennäköiseltä, että tutkitut skitsofrenian alttiusgeenit eivät selitä merkittävästi skitsofrenian sairastumisriskiä suomalaisessa väestössä.

Tutkimuksen toisessa ja kolmannessa osatyössä toistimme aluksi 352 perheen aineistossa skitsofreniakytkennän kromosomin 7 pitkän käsivarren alueelle. Seuraavassa vaiheessa hyödynsimme sairausdiagnoositiedon lisäksi erilaisiin kliinisiin sairauspiirteisiin ja välimuotoisiin ilmiasupiirteisiin—endofenotyyppeihin—perustuvia muuttujia yhteensä 290 neuropsykologisesti testatun perheen aineistossa. Havaitsimme kytkeytyvällä kromosomialueella sijaitsevassa *Reelin* (*RELN*) -geenissä sijaitsevan geenimerkin yhdistyvän sekä heikentyneeseen kognitiiviseen suoriutumiseen että vaikeampiin skitsofreniaoireisiin. Aikaisemman tutkimustiedon valossa *RELN*-geenin eri muodot vaikuttavat useiden keskushermoston kehityksellisten sairauksien taustalla. Tutkimuksessamme havaitun riskialleelin vaikutus toistui myös riippumattomassa aineistossa ja sen vaikutus oli voimakkaampi psykoottistasoiseen mielenterveyden häiriöön sairastuneiden keskuudessa. Vaikka merkittävää yhteyttä skitsofrenian kliiniseen diagnoosiin ei havaittu, tunnistettu riskialleeli oli yleisempi skitsofreniakirjon sairauksia sairastavien keskuudessa. Tuloksemme tukevat täten *RELN*-geenin merkitystä skitsofrenian taustalla ja erityisesti tautiin liittyvien piirteiden muokkaajana.

Aineistomme yhteensä 293 perheestä saatavilla olevia ilmiasumuuttujia hyödynnettiin myös tutkimuksemme viimeisessä osiossa. Tutkimusryhmämme on aiemmin havainnut sekä skitsofrenian että sen kognitiivisten endofenotyyppien kytkeytymisen kromosomien 2, 4 ja 5 pitkien käsivarsien alueelle. Valitsimme näiltä alueilta yhteensä 104 geeniä, joilla voi tunnettujen toimintojensa perusteella olla merkitystä skitsofrenian taustalla. Kyseisten geenien ja skitsofrenian yhteyden tutkimisessa hyödynnettiin sekä sairausdiagnoositietoja että kliinisiin sairauspiirteisiin ja kognitiivisiin endofenotyyppeihin perustuvia muuttujia. Havaitsimme lukuisia lupaavia yhteyksiä geeneihin, joista erityisen merkittäviä olivat *V-erb-a erythroblastic leukemia viral oncogene homolog 4* (*ERBB4*), joka yhdistyi vaikeampiin skitsofreniaoireisiin ja heikentyneeseen kielelliseen suoriutumiseen, sekä *Glutamate receptor, ionotropic, AMPA 1* (*GRIA1*), joka yhdistyi vaikeampiin skitsofreniaoireisiin.

Tutkimuksemme tukee näkemystä, että skitsofrenian monimuotoisuudesta johtuen kliininen sairausdiagnoosi voi olla tutkimuksellisena mittarina liian karkea kaikkien tautiin liittyvien alttiusgeenien tunnistamiseksi. Siten yksityiskohtaisemmat kliiniset sairauspiirteet ja välimuotoiset ilmiasupiirteet eli endofenotyypit voivat auttaa paitsi skitsofrenialle myös mahdollisesti muillekin psykiatrisille sairauksille altistavien geenimuotojen tunnistamisessa. Tuloksemme tukevat aikaisempaa arviota skitsofrenian geenitaustan monimuotoisuudesta. Tutkimuksemme perusteella skitsofrenian geneettinen tausta voi ainakin osin selittyä yksittäisten geenien vaikutuksella keskushermoston kehitykseen ja toimintoihin, mikä voi ilmetä erityisesti vaihteluna sairauteen liittyvissä piirteissä, kuten kognitiivisissa toiminnoissa. Tämän vuoksi tutkimustuloksemme rohkaisevat käyttämään monitekijäisen taudin alttiusgeenien tunnistamisessa erilaisia sairauteen liittyviä piirteitä ja välillisiä muuttujia, jotka mahdollistavat paitsi tutkimusaineiston tehokkaamman hyödyntämisen myös todennäköisesti sairauksien ilmiasuja muokkaavien geenien tunnistamisen.

Asiasanat: skitsofrenia, psykiatria, kytkentäanalyysi, assosiaatioanalyysi, perustajaväestö, endofenotyyppi

Contents

List of original publications

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

I Turunen JA*, **Peltonen JO***, Pietiläinen OP, Hennah W, Loukola A, Paunio T, Silander K, Ekelund J, Varilo T, Partonen T, Lönnqvist J, Peltonen L. The role of DTNBP1, NRG1, and AKT1 in the genetics of schizophrenia in Finland. Schizophr Res. 2007 Mar;91(1-3):27-36.

II **Wedenoja J**, Loukola A, Tuulio-Henriksson A, Paunio T, Ekelund J, Silander K, Varilo T, Heikkilä K, Suvisaari J, Partonen T, Lönnqvist J, Peltonen L. Replication of linkage on chromosome 7q22 and association of the regional Reelin gene with working memory in schizophrenia families. Mol Psychiatry. 2008 Jul;13(7):673-84.

III **Wedenoja J**, Tuulio-Henriksson A, Suvisaari J, Loukola A, Paunio T, Partonen T, Varilo T, Lönnqvist J, Peltonen L. Replication of association between working memory and Reelin, a potential modifier gene in schizophrenia. Biol Psychiatry. In press. Epub 2009 Nov 16.

IV **Wedenoja J**, Tuulio-Henriksson A, Suvisaari J, Loukola A, Surakka I, Varilo T, Mottaqui-Tabar S, Lahermo P, Serkkola E, Ripatti S, Ranki-Pesonen M, Lönnqvist J, Peltonen L, Paunio T. Study of regional candidate genes for cognitive endophenotypes and clinical features of schizophrenia in a population isolate. Submitted.

* These authors contributed equally to this work.

Publication I has appeared also in the academic dissertation of Joni Turunen (2007).

The Author's last name has changed after publication I.

Abbreviations

α3β1	Alpha-3-beta-1 integrin receptor
A	adenine
ACSL3	Acyl-CoA synthetase long-chain family member 3
AF	all Finland (outside internal isolate)
AKT1	V-akt murine thymoma viral oncogene homolog 1
ALL	whole sample
ANYPSY	psychotic disorders
AOO	age of onset
APOE	Apolipoprotein E
ApoER2	Apolipoprotein E receptor 2
C	cytosine
CAMK2A	Calcium/calmodulin-dependent protein kinase II alpha
CAMK2D	Calcium/calmodulin-dependent protein kinase II delta
CENTD3	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 3
CEPH	Centre d'Etude du Polymorphisme Humain
CFLAR	CASP8 and FADD-like apoptosis regulator
CHLC	Cooperative Human Linkage Center
CHRNA7	Cholinergic receptor, nicotinic, alpha 7
CI	confidence interval
cM	centiMorgan
CNS	central nervous system
CNV	copy number variation
COMT	Catechol-O-methyltransferase
CORESCH	schizophrenia
CSNK1A1	Casein kinase 1, alpha 1
CTRL	control sample
CVLT	California Verbal Learning Test
CYP27A1	Cytochrome P450, family 27, subfamily A, polypeptide 1
DAO	D-amino-acid oxidase
DAOA	D-amino acid oxidase activator
DGCR2	DiGeorge syndrome critical region gene 2
DISC1	Disrupted in schizophrenia 1
DISC2	Disrupted in schizophrenia 2
DLX1	Distal-less homeobox 1
DNA	deoxyribonucleic acid
DR	delayed recall of a story
DRD1	Dopamine receptor D1
DRD2	Dopamine receptor D2
DRD3	Dopamine receptor D3
DRD4	Dopamine receptor D4
DSM-IV	Diagnostic and Statistical Manual for Mental Disorders, fourth edition
DTNBP1	Dystrobrevin binding protein 1
DV	delayed visual recall
EF	executive functioning
EPHA4	EPH receptor A4
EPHA5	EPH receptor A5

ERBB4	V-erb-a erythroblastic leukemia viral oncogene homolog 4
EREG	Epiregulin
Fam	families
FN1	Fibronectin 1
G	guanine
GABA	gamma-aminobutyric acid
GABRB2	Gamma-aminobutyric acid (GABA) A receptor, beta 2
GAD1	Glutamate decarboxylase 1
GEE	Generalized Estimation Equation Model
GPRIN3	G protein regulated inducer of neurite outgrowth 3
GRIA1	Glutamate receptor, ionotropic, AMPA 1
GRID2	Glutamate receptor, ionotropic, delta 2
GRIN	N-methyl D-aspartate glutamate receptor
GRIN2B	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B
GRM3	Glutamate receptor, metabotropic 3
HBEGF	Heparin-binding EGF-like growth factor
HDAC3	Histone deacetylase 3
HDAC4	Histone deacetylase 4
HGNC	HUGO Gene Nomenclature Committee
hME	homogenous Mass Extend
HP	Haptoglobin
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A
HUGO	Human Genome Organisation
HWE	Hardy-Weinberg equilibrium
ICD-10	International Classification of Diseases, tenth edition
ID	identification
IL1B	Interleukin 1, beta
Ind	individuals
IR	immediate recall of a story
IRE	intrusive recall errors
IS	internal isolate
IV	immediate visual recall
KIF1A	Kinesin family member 1A
KLF7	Kruppel-like factor 7
L	verbal learning
LC	liability class
LD	linkage disequilibrium
LDe	recalling words after long delay
LOD	logarithm of odds
MAF	minor allele frequency
Mb	megabase
MHC	major histocompatibility complex
miRNA	micro ribonucleic acid
MLS	maximum likelihood score
MMN	mismatch negativity
mRNA	messenger ribonucleic acid
MT	mental tracking
mtDNA	mitochondrial deoxyribonucleic acid
MTHFR	5,10-methylenetetrahydrofolate reductase

NA	not available/not applicable/not assessed
NAP1L5	Nucleosome assembly protein 1-like 5
NCBI	National Center for Biotechnology Information
NDUFS1	NADH dehydrogenase (ubiquinone) Fe-S protein 1
NK	not known
NP	neuropsychological tests
NPL	non-parametric linkage
NR3C1	Nuclear receptor subfamily 3, group C, member 1
NRG1	Neuregulin 1
NRP2	Neuropilin 2
ns	not significant
OMIM	Online Mendelian Inheritance in Man
OPCML	Opioid binding protein/cell adhesion molecule-like
OPCRIT	Operational Criteria Checklist
OR	odds ratio
P300	event-related potential, 300 ms
P50	event-related potential, 50 ms
PAX3	Paired box 3
PCP	phencyclidine
PCR	polymerase chain reaction
PLXNA2	Plexin A2
PPP2R2B	Protein phosphatase 2, regulatory subunit B, beta isoform
PPP3CC	Protein phosphatase 3, catalytic subunit, gamma isoform
PRE	perseverative recall errors
pre-mRNA	precursor messenger ribonucleic acid
PRODH	Proline dehydrogenase (oxidase) 1
PS	processing speed
PURA	Purine-rich element binding protein A
RAD50	RAD50 homolog
RASGEF1B	RasGEF domain family, member 1B
RELN	Reelin
RGS4	Regulator of G-protein signaling 4
RNA	ribonucleic acid
RPGRIP1L	Retinitis pigmentosa GTPase regulator interacting protein 1 -like
rRNA	ribosomal ribonucleic acid
RS	replication sample
S	Stroop interference score
SANS	Scale for the Assessment of Negative Symptoms
SAPS	Scale for the Assessment of Positive Symptoms
SC	semantic clustering
SCHSPECT	schizophrenia spectrum disorders
SCID-I	Structured Clinical Interview for DSM-IV
SDe	recalling words after short delay
SEMA3A	Semaphorin 3A
SEPT11	Septin 11
SERPINE2	Serpin peptidase inhibitor, clade E, member 2
siRNA	small interfering ribonucleic acid
SLC18A1	Solute carrier family 18, member 1
SLC4A4	Solute carrier family 4, sodium bicarbonate cotransporter, member 4

SNP	single nucleotide polymorphism
SPEC2	CDC42 small effector 2
STR	short tandem repeat
T	thymine
TACR3	Tachykinin receptor 3
tagSNP	tagging single nucleotide polymorphism
TMT	Trail Making Test
TP53	Tumor protein p53
TPH1	Tryptophan hydroxylase 1
tRNA	transfer ribonucleic acid
TSNAX	Translin-associated factor X
U	uracil
UGT2A1	UDP glucuronosyltransferase 2 family, polypeptide A1
UTR	untranslated region
VCFS	Velocardiofacial syndrome
VeAb	verbal ability
VeAt	verbal attention
VeWM	verbal working memory
ViAt	visual attention
ViWM	visual working memory
VLDLR	Very-low-density lipoprotein receptor
WAIS-R	Wechsler Adult Intelligence Scale-Revised
WMS-R	Wechsler Memory Scale-Revised
ZCD2	CDGSH iron sulfur domain 2
ZNF804A	Zinc finger protein 804A

The gene names are in italics and protein names capitalized.

1 Introduction

Schizophrenia [OMIM 181500] is a severe mental disorder affecting about 0.5 to 1% of the population worldwide. It thus constitutes a significant burden for not only the affected individuals but also for the whole societies. Schizophrenia is characterized by positive symptoms, such as paranoia, hallucinations, and delusions, and negative symptoms, such as avolition, anhedonia, and thought poverty, as well as cognitive dysfunction affecting especially attention, working memory, and executive functioning. Treatment of these severe and usually highly disabling symptoms is challenging, as the present medication options lessen primarily the positive symptoms without any major improvement on the cognitive performance (Mueser et al. 2004, Tandon et al. 2009).

Currently, schizophrenia is considered a complex disorder of neurodevelopmental origin, its onset being contributed by both genetic and environmental factors. Despite the active research over the past decades, characterization of these predisposing factors has proven challenging (Rapoport et al. 2005, Keshavan et al. 2008). Despite the large number of chromosomal regions, candidate genes, and genetic factors suggested, their detailed roles at the population level remain mostly undetermined. The knowledge on specific underlying mechanisms could help, however, to understand the disorder pathophysiology, and to develop more accurate diagnostic procedures and treatment options.

In Finland, the founder effect arising from the small number of original settlers, the genetic bottlenecks causing random sampling to small subgroups, the genetic drift causing changes in gene variant frequencies, and the long-lasting isolation of the population have affected the Finnish gene pool (Norio 2003b). This population history has formed the basis for the enrichment of some rare genetic variants causative for nearly 40 rare monogenic disorders, together referred to as the Finnish Disease Heritage (Norio 2003a). Putatively, these same mechanisms may result in enrichment of disease alleles predisposing to complex disorders as well. This population feature, combined with the well-documented Finnish population history, extensive medical and parish registers, and relatively uniform cultural and social environment, makes the Finns an excellent study population for complex genetic studies. Furthermore, general attitude towards medical research is supportive in Finland, diminishing the possibility of distortion in population-wide sample collection (Peltonen et al. 2000). The schizophrenia family sample of the National Institute for Health and Welfare has already proven successful especially in studies of the *Disrupted in schizophrenia 1* (*DISC1*) gene, currently considered one of the most promising candidate genes for schizophrenia (Hennah et al. 2006).

The aim of this study was to search for novel genetic variants behind schizophrenia, as well as to further characterize the role of the variants already highlighted in studies of other populations.

2 Review of the literature

2.1 Schizophrenia

2.1.1 History

As none of the known ancient texts evidently refers to schizophrenia, no conclusive proof of its origin exists. The worldwide occurrence of schizophrenia supports, however, the view of its long-lasting presence among mankind (Gottesman 1991).

In 1809, John Haslam (1764-1844) in England, and Philippe Pinel (1745-1826) in France, made independently of each other the first clinically adequate descriptions of schizophrenia. Thereafter, both the number of studies describing the disorder with varying names and the number of diagnosed patients increased rapidly. In 1852, Benedict Morel (1809-1873) in France described for the first time schizophrenia with the term *démence précoce* (in Latin, *dementia praecox*). The term *dementia praecox* was later used in Germany by Emil Kraepelin (1856-1926), whose definitive work in categorization of the disorder symptoms and characteristics established the basis for the present diagnostic criteria. Later in 1908 in Switzerland, Eugen Bleuler (1857-1939) tried to introduce a new view on the symptom categories and renamed the disorder as *schizophrenia*. Although this term became later internationally accepted, the modern diagnostic systems are more based on Kraepelin's work (Gottesman 1991).

2.1.2 Diagnosis

Currently, two diagnostic systems are used: the International Classification of Diseases, tenth edition (ICD-10) (World Health Organization 1992), and the Diagnostic and Statistical Manual for Mental Disorders, fourth edition (DSM-IV) (American Psychiatric Association 1994). In Europe, ICD-10 is the official system in clinical practice, while DSM-IV is widely used in scientific research. In both criteria, the diagnosis is based on different categories and subcategories of the disorder characteristics, of which a certain minimum number need to be met. One of the main differences between the systems is the requirement for symptom duration for at least six months in DSM-IV but not in ICD-10. In practice, however, the differences between the systems are minor (Jager et al. 2004). As any specific clinical or laboratory test for schizophrenia remain non-existent, the diagnostic assessment is based on subjective symptoms experienced by the patient, objective symptoms observed by the physicians, and long-term information on the symptoms documented in the patient's medical records (Table 1).

Table 1. The DSM-IV diagnostic criteria for schizophrenia (American Psychiatric Association 1994).

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 Clinical features

Schizophrenia symptoms can be divided according to their emergence. Even before any actual symptoms, patients may develop some strange emotions in relation to self and/or environment (Huber et al. 1980). Later on, before the first acute phase of the disorder, patients usually experience some *prodromal symptoms* for a few days to several years. These include, for instance, neurotic symptoms (e.g., anxiety), mood symptoms (e.g., depression), cognitive symptoms (e.g., difficulties to concentrate), perceptional symptoms, apathy, sleep disturbances, and behavioral changes (suspiciousness, social withdrawal, etc.) (Yung et al. 1996).

In acute phase, the most prominent symptoms are the *positive symptoms*, the most characteristic features of schizophrenia. These include, for instance, hallucinations, delusions, paranoia, incoherence of behavior, and inconsistence of speech (Mueser et al. 2004, Tandon et al. 2009).

The acute phase is usually followed by stabilization of the disorder, which may typically last for several months. In this phase, the *negative symptoms* usually strengthen. These symptoms include, for instance, avolition, anhedonia, thought poverty, and speech impoverishment (Mueser et al. 2004, Tandon et al. 2009).

During the stabilization phase, patients are still prone to acute phases with positive symptoms dominating, and these two phases may alternate repeatedly. Later on, patients may attain a steady state in the course of the disorder, and may even be able to return back to work and/or other normal everyday activities. The overall performance usually remains, however, at a lower level than before the disorder onset, and typically shows no major improvement with time (Mueser et al. 2004, Tandon et al. 2009).

2.1.4 Endophenotypes

By definition, endophenotypes are intermediate factors between the clinical phenotype and the genotype. They are measurable but not directly detectable without "an aided eye" (Gottesman et al. 2003). Endophenotypes are assumed to involve same biological pathways as the disorder but to be related more closely to relevant gene effects and involve simpler etiological background (Gottesman et al. 2003, Braff et al. 2007). Therefore, a disorder endophenotype should (Gottesman et al. 2003):

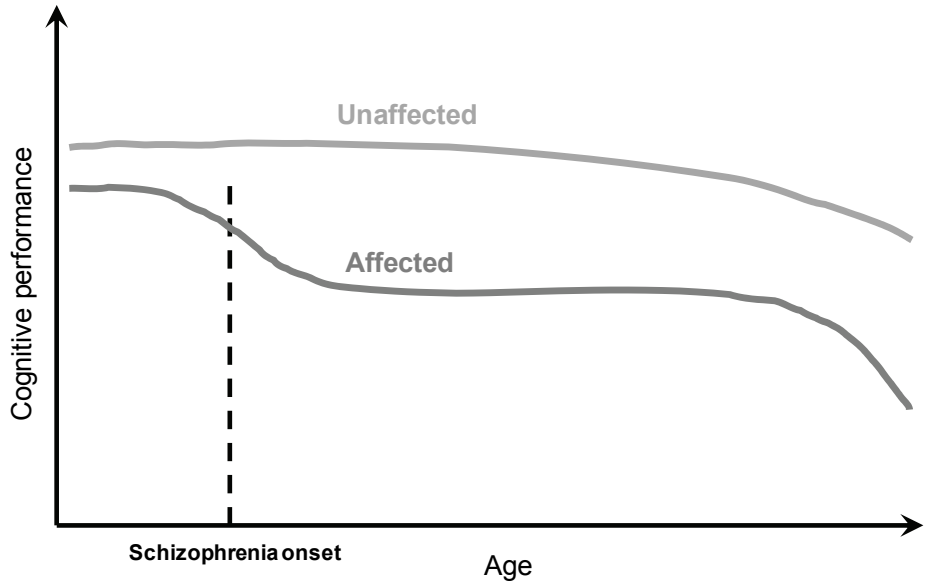
- associate with the disorder in the population
- be heritable
- be state-independent, that is, manifest independently of the disorder phase
- co-segregate with the disorder in families
- manifest in unaffected family members of patients at a higher rate than in the general population

In addition to positive and negative symptoms, schizophrenia patients usually have generalized cognitive impairment with deficits especially in attention, working memory, verbal learning and memory, information processing, and executive functioning (Heinrichs et al. 1998). Most of these deficits exist even before the first acute phase of the disorder, during which they rapidly deteriorate. Later, these deficits stabilize at a lower level than before the disorder onset and show no recovery thereafter (Heaton et al. 2001) (Figure 1).

These cognitive features appear also in milder forms in healthy relatives of the patients (Hoti et al. 2004), and are heritable (Tuulio-Henriksson et al. 2002, Greenwood et al. 2007), which implies their genetic background (Toulopoulou et al. 2007). Therefore, traits derived from neuropsychological tests measuring these cognitive functions have been suggested as valid endophenotypes for schizophrenia (Gur et al. 2007), as well as other psychotic disorders (Antila et al. 2007).

Other suggested endophenotypic traits for schizophrenia include different electrophysiological measurements of the brain activity, especially the event-related potentials P50 (Patterson et al. 2008), P300 (Bramon et al. 2004), and mismatch negativity (MMN) (Umbricht et al. 2005). Of these, the P50 and P300 are voltage deflections occurring roughly 50 ms and 300 ms after auditory stimulus as recorded in electroencephalography (Bramon et al. 2004). Although MMN resembles these, it is generated only if the stimulus is different from those preceding it (Umbricht et al. 2005). Other potential endophenotypic features include eye-tracking abnormalities (Kathmann et al. 2003), different pharmacological measurements, such as medication responses (Garver et al. 2000), and physical measurements of the brain concerning, for instance, cortical gray matter and both inter- and intrahemispheric white matter (Cannon et al. 2002, Tanskanen et al. 2008).

Figure 1. The average overall cognitive performance level in affected and unaffected individuals according to age. The performance level of an affected individual is usually slightly decreased even before the schizophrenia onset, and at the onset, the performance level usually drops quickly, showing no recovery thereafter (Heaton et al. 2001).



2.1.5 Pathophysiology

Although several brain abnormalities have been described in schizophrenia, these features are usually slight at the individual level. None of these abnormalities are specific for schizophrenia, therefore providing no support for diagnostics. Despite the observed brain abnormalities, the incidence of neurodegenerative disorders in schizophrenia patients is comparable with that of general population (Baldessarini et al. 1997).

At the macroscopic level of the brain in schizophrenia patients, the total cerebral volume is smaller, the total ventricular volume is larger, the total cortical grey matter is reduced, and the hemispheric asymmetry is reduced. There is no large-scale neuronal loss, however. As for the specific brain structures, most of the size aberrations concentrate in hippocampus, thalamus, amygdala, superior temporal gyri, and frontal and temporal cortex (Wright et al. 2000, Honea et al. 2005, Steen et al. 2006, Keshavan et al. 2008).

At the microscopic level of the brain in schizophrenia patients, the abnormal distribution of neurons especially in the layer II of the cortex and in the interstitial white matter, smaller size of the pyramidal cells, decreased number of the dendrites and interneurons, and reduced number and functionality of the oligodendrocytes emerge. In synapses, synaptic terminals have revealed only minor morphological changes (Harrison 1999, Harrison et al. 2005, Keshavan et al. 2008).

At the molecular level, several specific neurotransmitter systems seem to be affected, including dopaminergic neurons in the prefrontal cortex and striatum, glutamatergic neurons in the hippocampus and prefrontal cortex, and gamma-aminobutyric acidergic (GABAergic) and glutamatergic neurons in the dorsolateral prefrontal cortex (Harrison et al. 2005, Keshavan et al. 2008). However, whether these changes are primary features of the disorder or merely reflections of the fundamental neuropathology remains still somewhat unclear.

2.1.6 Epidemiology

The lifetime prevalence of schizophrenia varies from 0.2% to 1.2% worldwide with no significant differences between urban, rural, or mixed geographical regions. The prevalence seems to be lower in the least developed countries, although the possibility of an underlying statistical bias based on, for instance, under-diagnosing the disorder, must be taken into account. Additionally, the prevalence among migrants is 1.8 times higher when compared to natives (Saha et al. 2005). Interestingly, schizophrenia prevalence has shown no significant changes over time despite the schizophrenia patients having fewer offspring than the general population (McGrath et al. 1999).

Although schizophrenia is equally prevalent in men and women (Saha et al. 2005), men tend to have more severe course of the disorder (Castle et al. 1995). In addition, the average age of onset varies between the genders, being early twenties in men, and mid-to-late twenties in women, with the second peak of onset in women being at the age of menopause (Castle et al. 1995).

Heritability is defined as the proportion of phenotypic variation in the population which is attributable to genetic factors (Visscher et al. 2008). For schizophrenia, the heritability estimates among twins are as high as 83% to 88%, implying strong genetic background

(Cardno et al. 2000). This is supported by adoption studies revealing risk connection between biological relatives but not adoptive relatives of the schizophrenia patients (Tienari et al. 2004). Although close relatives of schizophrenia patients have substantially elevated risk for schizophrenia, and the amount of affected siblings elevates the risk (Hovatta et al. 1997), the risk diminishes rapidly in more distant relatives (Gottesman 1991, Tsuang 2000) (Table 2). Additionally, the majority of schizophrenia patients belong to families with no known history of the disorder or other psychotic conditions (Gottesman 1991, Tsuang 2000).

In addition to schizophrenia itself (the "core schizophrenia"), the incidence of so-called schizophrenia spectrum disorders is elevated among the close relatives of schizophrenia patients (Kendler et al. 1993a, Kendler et al. 1993b, Kendler et al. 1993c, Kendler et al. 1993d). Although different criteria exist concerning the disorders belonging to the spectrum, the broad definition used by our research group comprises schizoaffective, schizophreniform, delusional and brief psychotic disorders, and schizoid, schizotypal and paranoid personality disorders, as well as psychotic disorder not otherwise specified. The view that all these disorders likely share at least some factors in their pathophysiological and genetic background is further supported by the Finnish Adoptive Family Study of Schizophrenia, showing broadly dispersed liability for several schizophrenia-related disorders (Tienari et al. 2003).

The genetics of schizophrenia is discussed in chapter 2.4.

Table 2. Morbid risks of schizophrenia. Adapted from (Tsuang 2000).

Relationship	Shared genes	Risk
General population	NA	1%
Spouses of patients	NA	2%
Third-degree relatives	12.5%	
• First cousins		2%
Second-degree relatives	25%	
• Uncles/aunts		2%
• Nieces/nephews		4%
• Grandchildren		5%
• Half-siblings		6%
First-degree relatives	50%	
• Parents		6%
• Siblings		9%
• Children		13%
• Siblings with 1 schizophrenic parent		17%
• Dizygotic twin		17%
Monozygotic twin	100%	48%
Children with 2 schizophrenic parents	100%	46%

Abbreviations: NA, not applicable

2.1.7 Environmental risk factors

The risk of schizophrenia seems to be increased in conjunction with several obstetric complications. These include complications in pregnancy, abnormal fetal growth and development, and complications in delivery, altogether increasing the schizophrenia risk twofold (Cannon et al. 2002). Other risk factors include maternal infections, especially influenza (Mednick et al. 1994) and other respiratory infections (Brown et al. 2000b), polio (Suvisaari et al. 1999), and rubella (Brown et al. 2000a), as well as prenatal exposure to famine (St Clair et al. 2005, Xu et al. 2009). Additionally, large sibship size (≥4), young maternal age, and early age of onset of the parent with schizophrenia seem to increase the risk (Haukka et al. 2004).

After birth, known environmental risk factors include infections of the central nervous system (Rantakallio et al. 1997), malnutrition (Wahlbeck et al. 2001), as well as abuse and maltreatment during childhood (Ellason et al. 1997, Read et al. 1999). Later, cannabis use is known to increase the risk twofold (Arseneault et al. 2002).

None of these environmental factors increase, however, the risk for schizophrenia specifically.

2.1.8 Treatment

On average, schizophrenia causes considerable shortening of the impending lifespan for secondary reasons (Saha et al. 2007). Although relatively large proportion of these are unnatural causes, such as the notably elevated suicide risk (Kooyman et al. 2007), the majority are natural causes, such as cardiovascular diseases, though possibly at least partly being consequences of, for instance, medication side-effects, such as weight gain (Saha et al. 2007). As the clinical picture and course of schizophrenia varies, treatment options need to be evaluated individually (Barrowclough et al. 1999), and delays in commencement of the treatment debilitate recovery (Marshall et al. 2005, Marshall et al. 2006).

The present pharmacological intervention is mostly based on antipsychotic medication, which typically lessens positive symptoms effectively but has much smaller effect on negative and cognitive symptoms (Keefe et al. 2007). The major challenges are that the actual functional disability of the patient correlates with the severity of the negative symptoms and the cognitive impairment but not with the positive symptoms (Elvevag et al. 2000), and that relatively large proportion of the patients experience relapses when taking the medication (Kissling et al. 1999).

The majority of schizophrenia patients are currently treated with second-generation antipsychotics, a highly heterogeneous group of drugs. The most widely used of these are clozapine, olanzapine, risperidone, and quetiapine, of which only clozapine is associated with reduced mortality when compared with the first-generation antipsychotic perphenazine (Tiihonen et al. 2009).

In addition to medication, different psychosocial therapies are recommended as they assist the functional recovery, for instance, in social life and work (Kern et al. 2009).

2.2 Human genome

2.2.1 Structure

The human genome is comprised of about three billion nucleotides in the form of double-stranded deoxyribonucleic acid (DNA) divided into 22 pairs of autosomal chromosomes and sex chromosomes denoted as X and Y. Of these, women carry two X chromosomes, and men both one X and one Y chromosome. Half of the chromosomes, including one of the sex chromosomes, are inherited from the mother and another half from the father. In addition to the chromosomes, the mitochondria of the human cells contain a small amount of DNA, mitochondrial DNA (mtDNA), which is inherited solely from the mother (International Human Genome Sequencing Consortium 2004).

DNA, the storage form of the hereditary information, is packed in the nuclei of cells. All the human cells, excluding the gametes, contain the whole genetic information which is expressed differently depending on the type or function of the cell. In DNA, bases adenine (A), thymine (T), cytosine (C), and guanine (G) form nucleotide pairs (A and T; C and G) between the opposite DNA strands with backbones involving sugars and phosphate groups. The DNA strand has directionality due to its chemical composition. The so-called 5' end of the DNA backbone contains a hydroxyl group, and the so-called 3' end contains a phosphate group. New monomers are added to the backbone via dehydration reaction which can only use the 5' end hydroxyl as nucleophile, thus the DNA is always read in 5' to 3' direction (Gerstein et al. 2007).

The genetic code is utilized and read in groups of three nucleotides called codons. Each of the 64 different codons correspond to one of the 20 amino acids, the building blocks of proteins, or mark the ending positions, stop codons, of the DNA read frame. The human genome contains altogether 20 000 to 25 000 genes. Only about 1.5% of the total DNA comprises all the protein-coding sequences, however. The role of the non-coding regions is still very much unknown, but supposedly involves many regulatory sites in addition to so-called "junk" DNA with no known function (International Human Genome Sequencing Consortium 2004).

When the genes are expressed, the corresponding DNA strand is read and copied in the process called transcription. The forming messenger ribonucleic acid (mRNA), at the first stage called precursor mRNA (pre-mRNA), is comprised of the same nucleotides as DNA, with the exception of uracil (U) replacing thymine. Unlike DNA, RNA is a single-stranded structure. When the required reading frame of the DNA is copied, parts corresponding to the non-coding DNA of the newly formed pre-mRNA are spliced out, leaving only the nucleotides coding for amino acids in the so-called mature mRNA. The mRNA is then transferred from the nucleus to cytoplasm, where it is read in ribosomes one codon at a time in the process called translation, and corresponding amino acids are added by the transfer RNA (tRNA) in chain to form the protein structure (Black 2003).

In addition to mRNA and tRNA, human cells involve ribosomal RNA (rRNA), part of the ribosomal structure, and different microRNAs (miRNAs) and small interfering RNAs (siRNAs), which are small RNA molecules of 20-25 nucleotides taking part in post-transcriptional regulation of gene expression (Ghildiyal et al. 2009).

2.2.2 Genetic variation

Genetic variation can be divided in recombinations and structural variations of the DNA sequence. In *recombination*, the DNA strand is cut and then united again at different location. Recombinations occur between the chromosome pairs in every meiosis, that is, in formation of gametes with halved amount of chromosomes. Therefore, recombination is a basic source of genetic variation in the offspring. These recombinations have basically no effect on the function of DNA as they just divide the inherited genetic material. Additional recombinations may occur, however, and depending on the position of the cut, they may or may not affect DNA function. If, for instance, the DNA strand is cut under or very close to a gene, the transcription of that gene is practically always distorted or prevented (Coop et al. 2007). Genetic distance along chromosome in which a recombination is statistically supposed to occur is called Morgan, however, in genetic analyses a smaller quantity centiMorgan (cM) is usually used. It equals to 1% chance of recombination and corresponds to roughly one million nucleotides in length, that is, one megabase (Mb) (Barzel et al. 2008).

Structural variations can be divided into those altering the DNA sequence length and those changing the sequence content without affecting the length. Furthermore, structural variations can be classified into *mutations* and *polymorphisms*. The central characteristic of a mutation is that it affects the function of DNA and thus cosegregates with some feature, for instance, disorder status, whereas polymorphisms appear functionally neutral. If the mutation has reduced penetrance, however, its effect may not always manifest (Frazer et al. 2009).

The variations altering the sequence length are called *deletions*, in which one or more nucleotides are removed from the sequence, and *insertions*, in which one or more nucleotides are added into the sequence. Depending on the length of the deletion/insertion it may remove/add one or more codons as whole, the others remaining unaffected. But if the deletion/insertion length is not divisible by three, it changes the DNA reading frame entirely from the deletion/insertion point forward (Hastings et al. 2009) (Table 3).

A special form of insertions are *duplications*, in which even whole genes or large parts of the chromosome are duplicated, usually as a result from an error in recombination. Because duplicated genes are known to undergo mutations more often than original genes, they may have evolutionary effects (Zhang 2003). Duplications and large deletions occur most often in repetitive intronic elements of the genes and are designated as *copy number variations* (CNVs) (Hastings et al. 2009) (Table 3).

Translocations, in which larger chromosome regions change their positions reciprocally, may be balanced, in which the exchange of genetic material is equal, or unbalanced, in which the exchange of genetic material is unequal, that is, some chromosomal region(s) are duplicated or deleted. Thus, the translocations may interfere the function of the regional genes, although balanced translocations are usually functionally neutral (Barzel et al. 2008).

In *inversions* the order of the nucleotides is reversed for a specified length of the DNA sequence, varying from only a few nucleotides to large parts of the whole chromosome. Similarly to translocations, also inversions may be unbalanced, and balanced inversions may remain functionally neutral (Frazer et al. 2009).

A variation conserving the DNA sequence length is called *point mutation*. It involves one changed nucleotide or nucleotide pair, thus called single nucleotide polymorphism (SNP), which potentially changes the amino acid encoded by the corresponding codon. Although majority of these point mutations have no effect on the protein structure and are thus called silent mutations, emerging evidence supports that also they may play a role in gene expression regulation due to alterations in the secondary mRNA structure, causing changes in the transcription. In addition, although the amino acid specified by the altered codon may remain the same, the amounts of different tRNAs corresponding to the same amino acid may differ, potentially affecting the transcription speed (Chamary et al. 2006) (Table 3).

Table 3. Examples of different human genetic variants. For deletions, duplications, insertions, and copy number variations, the size of the variant may vary between a few nucleotides, as presented here, to hundreds of thousands of nucleotides. The variants are shaded and repetitive elements underlined.

Insertion/	GTTCAAGACTAGCATGGCCAAGAT
Deletion	GTTCAAGAC---CATGGCCAAGAT
Duplication/	GTTCAAGACTAGCAAGACTAGCATGGCCAAGAT
Copy number variation	GTTCAAGACTAGCATGGCCAAGAT
Inversion	GTTCAACCATGCTAGTCCCAAGAT
	GTTCAAGACTAGCATGGCCAAGAT
Point mutation/	GTTCAAGACTAGCATGGCCAAGAT
Single nucleotide polymorphism	GTTCAAGACTATCATGGCCAAGAT
Polymorphic site/	GTTCAAGACTAGCGCGCGCGCGCAAGAT
Short tandem repeat	GTTCAAGACTAGCGCGCAAGAT

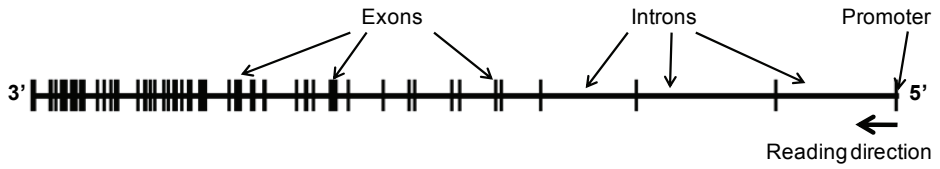
2.2.3 Gene structure and function

Genes are the basic units of heredity, comprising information of maintaining the cells and their functions. Overall, human DNA carries two copies of every gene, one inherited from the mother and another from the father. The traditional definition of a gene is a protein-coding unit of the DNA sequence. Based on the present knowledge, however, this definition is no more sufficient as genes may, for instance, encode for RNA structures not being translated but used for regulatory actions (Gerstein et al. 2007).

The gene starts with a *promoter*, in the 5' end of the gene, which is a position recognized by the transcription machinery in the nucleus in the beginning of the transcription. The promoter is followed by a variable number of alternating *exons* and *introns*. Exons are the parts of the DNA sequence which encode for the amino acid structure of the translated protein. In addition, the last coding exon contains the *stop codon* which ends the transcription. Introns, being longer than the exons, supposedly comprise different regulatory functions, for instance, *enhancer elements* binding activator and repressor proteins. In addition, the highly conserved regions at the ends of the introns are required in the mRNA splicing, in which the introns are spliced away from the pre-mRNA,

likewise part of the 3' untranslated region (UTR) which may follow the last exon (Gerstein et al. 2007) (Figure 2).

Figure 2. Schematic of a gene structure, here the *Reelin* (*RELN*) gene on chromosome 7q22 shown as an example. The exons are emphasized for visualization purposes.



2.2.4 Gene expression regulation

All the gene expression regulation beyond the DNA structure itself is called epigenetic regulation. At the DNA level, *histone modifications* affect the basic properties of the DNA, for instance, gene expression and sequence repair. The histones are protein structures around which the DNA strand is coiled, compacting it about 40 000-fold (Grewal et al. 2007). In *DNA methylation*, methyl groups attach to the DNA strand and typically diminish or prevent transcription of the gene under the sequence. The DNA sequence comprises numerous GC-rich areas which are prone to methylation (Robertson 2005). Furthermore, different *activator* and *repressor proteins* can bind to enhancer elements typically in the introns of the genes and initiate or decrease the gene expression, respectively (Farnham 2009).

At the RNA level, *alternative splicing* of pre-mRNA results in different mRNA sequences and thus different protein isoforms. Over 80% of human genes are alternatively spliced, most commonly in the process called exon skipping, in which some exon or exons are included in the mRNA in some condition(s) and spliced away in some other(s). Diverse regulatory mechanisms for alternative splicing include, for instance, different activator and repressor proteins which bind to pre-mRNA and affect the locations of the splice site junctions (Black 2003, Matlin et al. 2005). Additionally, *miRNAs* and *siRNAs* can bind to the 3' UTR of an mRNA sequence, preventing its translation and therefore silencing the gene in a post-transcriptional manner. This phenomenon has provided sophisticated methods to study gene functions by silencing genes after transcription (Ghildiyal et al. 2009).

2.2.5 Genetic disorders

Approximately 99.9% of the human genome is identical between individuals. Therefore, only a minor part of the DNA sequence constitutes all the genetic variation in the mankind, including the disorder-related variants (Reich et al. 2002).

Monogenic disorders, often called as Mendelian disorders after Gregor Mendel (1822-1884), a famous Austrian scientist and priest, are caused by a defect in a single gene and follow simple rules of inheritance. In *dominant disorders*, a single copy of mis- or unfunctional gene causes the disorder, in which case the gene may be inherited from

either parent with the exception of the sex chromosomal genes. In *recessive disorders*, both gene copies must be mis- or unfunctional to cause the disorder, and thus, to be inherited from both parents. In these cases, parents are usually unaffected carriers of a single affected gene. As a rare exception are the genes with only one active copy due to a process called parental imprinting, in which only the gene copy inherited from the mother or father is expressed, and thus, a single mutation in that active gene is sufficient to cause the disorder (Antonarakis et al. 2006).

The vast majority of the disorders are, however, *multifactorial*. In these cases, affected individuals usually have several genetic alterations, and different environmental factors play a role in the pathogenesis. Typically the disorder risk varies, and it may expose gradations depending on the affected genes, the number of them, and/or the amount and level of the environmental strain. Multifactorial disorders are often called complex disorders due to the diversity of their background (Frazer et al. 2009).

2.2.6 Generalist and modifier genes

In addition to the variants directly increasing the disorder liability, so-called *generalist genes* may affect different characteristics in the general population regardless of the affection status. They may, however, also alter both the disorder liability and its features in a secondary manner. In addition, one gene may affect several individual characteristics, called pleiotropy, and on the other hand, several individual genes may affect the same characteristic, called polygenicity (Kovas et al. 2006). For cognitive functions, their high heritability, up to 80%, and established correlations between different genetic associations with different cognitive domains support the generalist gene hypothesis (Butcher et al. 2006).

Instead, the *modifier genes* may show stronger effects in combination with other genetic variants directly increasing the disorder liability (Nadeau 2001). As an important difference compared to generalist genes, modifier genes supposedly show only slight effects among unaffected individuals (Fanous et al. 2001). However, different hypothesized modifier gene subtypes vary in their effects on liability to different disorder subtypes, disorder characteristics, and features in unaffected individuals (Fanous et al. 2005). Overall, several reported associations between different candidate genes and features of psychiatric disorders without association to clinical diagnosis support the view that genetic variants could modify the disorder characteristics without altering the liability itself (Fanous et al. 2005).

2.3 Genetic mapping of complex disorders

2.3.1 Study samples

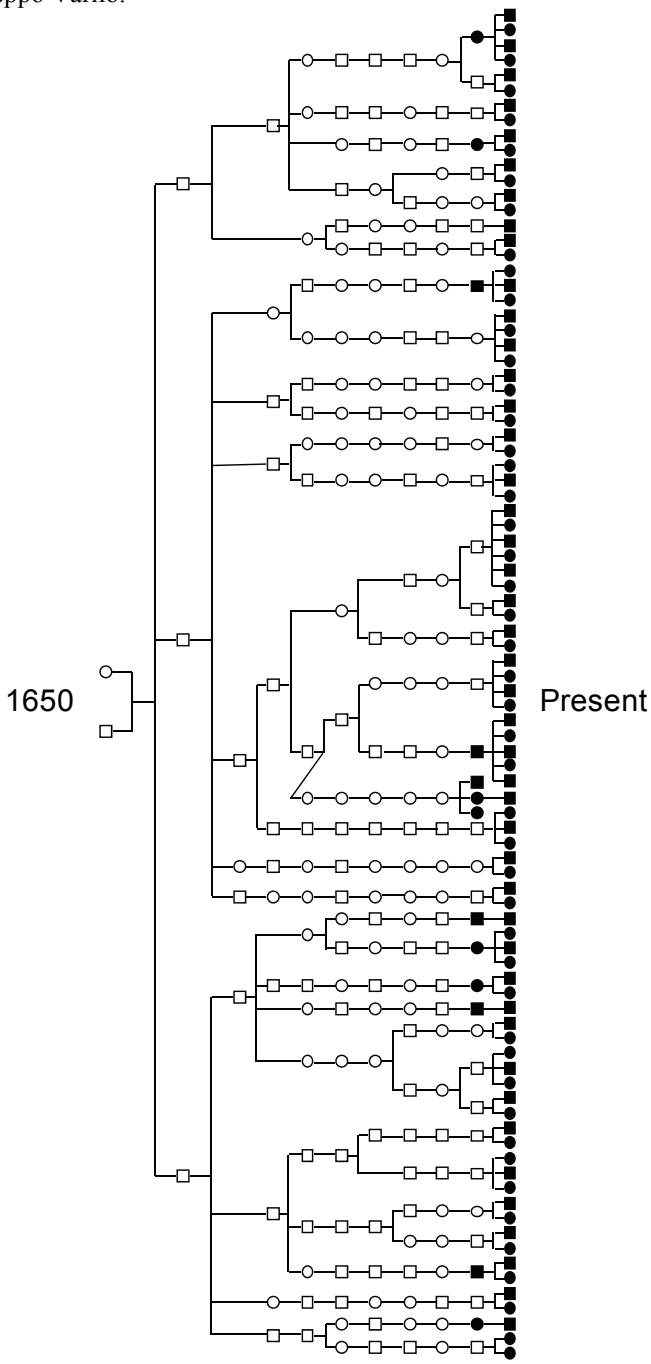
Family samples usually involve enriched nuclear families and/or pedigrees with multiple affected individuals. This diminishes genetic variation and favors identification of causative risk variants. Additionally, segregation of variants can be monitored through generations. A specific subtype of family samples is a trio sample comprising parents and their single offspring. Typically, family samples allow identification of rare variants with a strong effect, and are used especially in linkage analysis (Pritchard et al. 2002) (Figure 3).

Case-control samples comprise affected individuals and control individuals matched to the affected ones in all other aspects than the disorder or trait under investigation. The basic simplicity of this series favors collection of large sample sizes relatively easily, allowing proper statistical power. Typically, case-control samples allow identification of common variants with a small effect, and are used especially in association analysis (Palmer et al. 2005).

Twin samples involve mono- and dizygotic twins, either equal or opposite in terms of disorder status, called concordant and discordant pairs, respectively. As the twin samples settle somewhere between the family and case-control samples, they allow some special research frames, for instance, the estimation of disorder or trait heritability based on the comparison of concordance rates in monozygotic and dizygotic twins for the investigated trait (Cardno et al. 2000).

Population cohorts comprise all the people who have a common characteristic, for instance, are born in a defined period of time, in which case called as birth cohort. Cohorts are typically used in longitudinal follow-up studies, allowing especially the estimation of the impact of environmental factors on the disorder. Another useful purpose for population cohort is to investigate the impact of identified risk factor(s) at the general population level (Hattersley et al. 2005). As the cohort studies tend to require considerable financial investments, they are usually carried out only in developed countries, and considering birth cohorts, may require several decades of follow-up (Welham et al. 2009).

Figure 3. Schizophrenia pedigree from an internal isolate (IS) of Finland with all the affected individuals (filled symbols) sharing common ancestors dating back to 1650 (Hovatta et al. 1999). Here, only the connecting relatives are shown. Pedigree figure courtesy of Teppo Varilo.



2.3.2 Population isolates

In all study samples, population stratification, that is, the study sample involving subpopulations with different allelic frequencies, may lead to false positive discoveries (Cardon et al. 2003), or on the other hand, mask the effects of true positive signals (Helgason et al. 2005). To overcome this, population isolates with limited allelic variation and more uniform background may be utilized, such as the Finns (Peltonen et al. 2000).

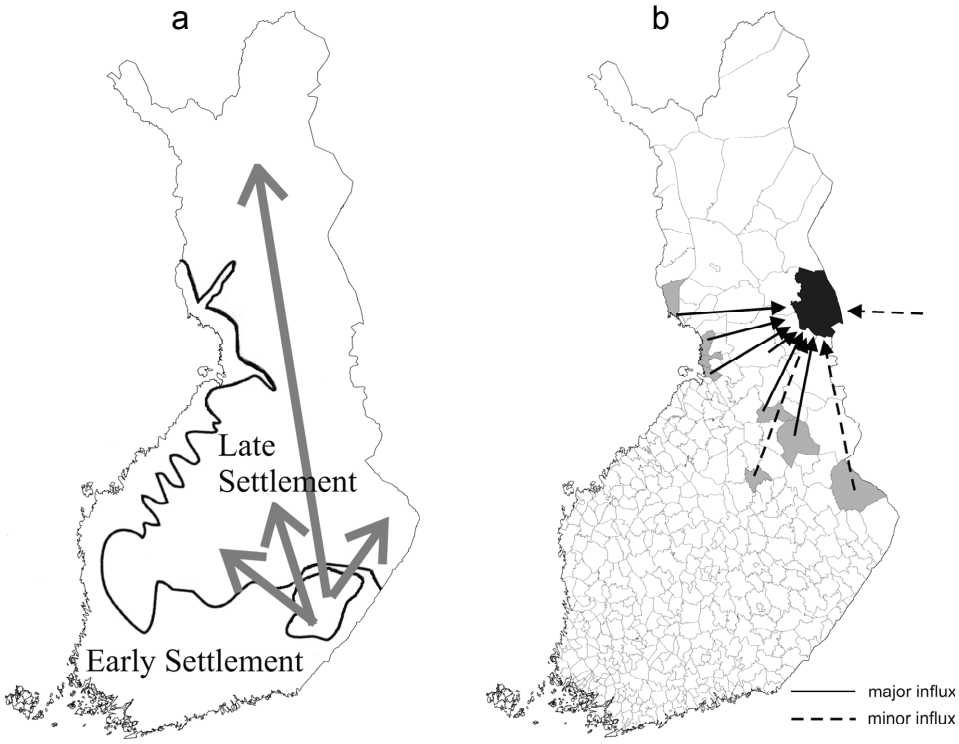
The coastal regions of Finland were inhabited after the last glacial period, approximately 10 000 years ago, by small individual groups following the melting ice. Presumably, additional migration waves arrived about 4000 and 2000 years ago mainly from South Russia and Central Europe, respectively. Despite the supposed northern migration by some of the earliest settlers, the possible ancestors of the Saami population, no major migration to northern and eastern Finland occurred before the 1500s. At that time, small individual groups especially from South Savo began moving inland from the early settlement region (Figure 4a). The incoming settlers forced the Saami to retreat further north, and apparently, no major genetic admixture occurred between these subpopulations. The *genetic drift*, random sampling of gene variants into these settler groups and changes in their frequencies over time, and *genetic bottlenecks* caused by, for instance, the great famine during 1696-1698 killing about third of the population, have affected the Finnish gene pool. Importantly, due to long-lasting isolation, the expansion of the Finnish population has resulted mostly from population growth, with only minor external migration. Due to this *founder effect*, arising from small number of the original settlers and causing reduced genetic variation, the Finns and similarly isolated populations are often called *founder populations* (Varilo 1999).

Because of these effects, genetic variants predisposing to altogether 36 monogenic disorders, referred to as the Finnish Disease Heritage, have enriched in Finland (Norio 2003a, Norio 2003b, Norio 2003c). Analogous enrichment of alleles predisposing to complex disorders is plausible (Peltonen et al. 2000), supported by the limited genetic diversity (Sajantila et al. 1996) and high interdependence, that is, linkage disequilibrium (LD) between genetic markers in Finns (Service et al. 2006). These features, combined with the well-documented population history, extensive medical and parish registers, and relatively uniform cultural and social environment, supports the usability of Finnish samples in genetic studies (Varilo 1999).

The use of population isolates comprises some caveats, however. Even some relatively common genetic disorders may be almost absent in an isolate. For instance, cystic fibrosis and phenylketonuria are rare among Finns (Kere et al. 1994, Guldberg et al. 1995). On the other hand, frequencies and thus impact of different alleles may differ between different populations, restricting the generalizability of the study results (Palmer et al. 2005). Furthermore, even population isolates may comprise internal genetic substructures which need to be taken into account in genetic studies (Salmela et al. 2008, Jakkula et al. 2008). Although by the end of 1600s nearly the whole Finland was inhabited, the inhabitant groups remained small and surprisingly isolated even until World War II. In Finland, this has influenced not only the genetic substructures but also regional prevalences of many disorders. For instance, in an internal isolate (IS) in the northeastern part of Finland, the lifetime risk of schizophrenia is 3.2%, compared to 1.1% in the rest of Finland (Hovatta et al. 1997) (Figure 4b). This suggests that different

risk variants and/or differences in their frequencies may affect the liability in and outside the isolate (Varilo 1999).

Figure 4. a) Internal migration of inhabitants in Finland in the 1500s. Movement of several small independent individual groups from the early (before 1500s) to the late (beginning from 1500s) settlement region caused genetic bottlenecks, resulting in internal genetic isolates. b) The inhabitation in the 1600s of an internal isolate (IS) with a higher than average incidence of schizophrenia (Varilo et al. 2000). Maps courtesy of Teppo Varilo.



2.3.3 Traits

Qualitative traits are dichotomous classifications. Typically, a study sample is divided into individuals with or without the investigated trait, usually disorder status based on the diagnostic information. Qualitative traits are widely used especially in the case-control studies due to their simplicity in data collection and statistical analysis. The disadvantage is that they may be too cursory to allow proper description of the investigated disorder or trait, and may not allow sufficient statistical power especially in the analysis of complex traits (Balding 2006).

Quantitative traits are continuous variables typically based on different measurement information. In schizophrenia research, for instance, many of the endophenotypic traits are continuous variables. In the analysis, continuous variables are usually required to follow the normal distribution. Additionally, the distances of the trait values from each other must be equal. In the analysis of both qualitative and quantitative traits, the trait status of some individuals may remain unknown, meaning that these subjects provide information only on the genetic variant frequencies (Balding 2006, Mackay et al. 2009).

Other traits include, for instance, *class variables* which settle somewhere between the qualitative and quantitative traits when compared with data handling and statistical power in the analysis. In this arrangement, study subjects are assigned in different classes on the grounds of, for instance, phenotypic clusters. If the classes are based on measurement information and their number is large enough, usually at least seven, they may alternatively be treated in the analysis as quantitative variables (Balding 2006).

2.3.4 Genetic markers

Of the currently used markers, the *single nucleotide polymorphisms* (SNPs) are the most frequent point mutations in the genome. Although majority of these, called synonymous SNPs, are assumed to be genetically neutral as they do not change the corresponding amino acid, at least some of them may possess a role in the gene expression regulation via alterations in the mRNA structure (Chamary et al. 2006). While single exonic or intronic SNPs are typically relatively uninformative by themselves, their relatively simple detection methods, and their total amount of over four million in the human genome make them feasible in the genetic mapping (Kidd et al. 2008). The current chip-based genotyping technologies allow hundreds of thousands of SNPs to be genotyped simultaneously.

Due to their density, SNPs on the same chromosome are usually inherited together in combinations, that is, *haplotypes*. The haplotypes have higher information content in the analysis than the individual markers, since the individuals sharing a variant affecting the investigated disorder or trait supposedly share also other nearby alleles, that is, the same haplotype harboring the variant. The chromosomal regions with low number of recombinations and thus relatively stable haplotype structures are called haplotype blocks. The haplotype block structures and, on the other hand, regions with elevated rate of recombination—so-called recombination hotspots—are partly shared between different populations, however, only a minority of them are exactly the same in all populations (Jakobsson et al. 2008). The haplotype phenomenon is also utilized in selection of so-called tagging SNPs (tagSNPs) which are in high LD with other SNPs in the same haplotype block. The LD is usually measured as co-variance between markers, denoted as D', or squared correlation coefficient, denoted as r^2. Theoretically, tagSNPs provide information on those other SNPs as well and thus allow the extraction of maximal information with a minimal set of markers (de Bakker et al. 2005). Despite some controversy (Terwilliger et al. 2006), this phenomenon is nowadays widely accepted and utilized in the marker selection (International HapMap Consortium 2005) (Table 3, Figure 5, Table 4).

The *short tandem repeats* (STRs) are polymorphic sites in the DNA sequence, consisting of repeating units of typically 2 to 10 nucleotides in length. The about 10 000 known STRs locate mostly on non-coding DNA regions. The STRs reveal elevated rate of

mutation due to being prone to errors in DNA replication and recombination. This allelic variability makes the typical STRs highly polymorphic and thus informative in the analysis, especially in family samples. On the other hand, the somewhat laborious genotyping has reduced their current use (Bailey et al. 2006) (Table 3).

In *sequencing*, the order of all nucleotides within the sequence region of interest is determined. Traditionally, this has required significant computational and financial efforts, but the technological progression will supposedly allow its more extensive use even in the near future. The sequencing of the whole human genome has set the basis for all the modern genetic research (Lander et al. 2001, Venter et al. 2001).

Figure 5. LD between SNPs and estimated haplotype blocks in *Reelin* (*RELN*) gene according to HapMap CEPH genotype data (all SNPs with minor allele frequency ≥0.10) (International HapMap Consortium 2005). The SNP positions are indicated with lines, and the estimated haplotype blocks are presented as triangles underneath the gene at the top. The darker the coloring in the LD diagram, the stronger the LD between the SNPs. Tagging SNPs may be estimated and selected from the SNPs in high LD with each other, and they are expected to reflect the effects of those other SNPs as well.

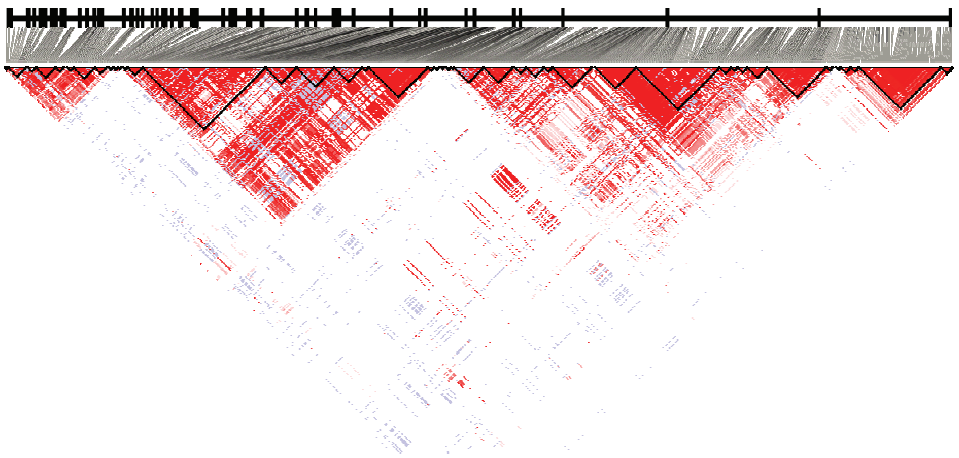


Table 4. An example of tagSNPs in three haplotypes from a haplotype block. The tagSNPs are shaded, and are together sufficient to identify the haplotype structures.

Haplotype 1	G T C A C A T A A C G T C G G
Haplotype 2	G T C A T A G A A C C T C C G
Haplotype 3	G G C G T C G A A C G A C C G

2.3.5 Statistical analysis

In *linkage analysis*, inheritance of genetic markers is followed up and analyzed against trait information. In general, linkage analysis tests if a genetic marker is inherited with the trait more often than would be expected by chance. If the analyzed marker does not itself affect the investigated disorder or trait—thus, is not the causative variant—it should reflect the effects of the causative one if they are located relatively close to each other and, therefore, recombination occurs only rarely between them. Linkage analysis is especially suitable for detection of rare high-risk variants. The linkage signal is usually expressed as logarithm of odds (LOD), which is the logarithm of the ratio of probabilities of obtaining a result with a specified degree of linkage and obtaining the same result with no linkage. For genome-wide analysis, LOD score of 3.3 is usually interpreted as statistically significant (Lander et al. 1995). In *parametric linkage analysis*, the causative variant frequency, penetrance (probability of the causative variant actually causing the disorder or having effect on the trait), and phenocopy rate (probability of individuals sharing the disorder status or trait independently of the causative variant) need to be estimated in advance. In contrast to monogenic disorders, these cannot usually be estimated exactly for complex disorders. Although *non-parametric linkage analysis,* not using any assumptions on the disorder or trait inheritance, may be utilized to overcome this (Goring et al. 2000), the location of the linkage signal in complex disorders with equivocal inheritance is prone to fluctuation, making the positional mapping challenging (Roberts et al. 1999, Altmuller et al. 2001).

In *association analysis*, genetic marker allele frequencies are analyzed against trait information. In general, association analysis tests if the marker allele is more frequent among the individuals with the investigated trait than would be expected by chance. Although the association analysis may enable accurate location of the predisposing variant, its sensitivity diminishes quickly with distance. Association analysis is especially suitable for detection of common variants. The association signal is usually expressed as P-value, which is the probability of obtaining a result at least as extreme as the one observed when the null hypothesis—for instance, that the investigated genetic marker has no effect on the disorder status or trait—is actually true. For genome-wide analysis, a P-value of 0.00005 has been suggested as statistically significant (Colhoun et al. 2003). The usual problems with association analysis include, for instance, density and relatedness of the markers, multiple testing, and population stratification (Cardon et al. 2003, Weiss et al. 2000). However, in detection of variants with modest effects the association analysis is superior compared to linkage analysis (Risch et al. 1996).

In both of these analyses, additional covariates may be included, which are additional factors affecting the analyzed trait. Their inclusion may assist in the detection of predisposing variants and, on the other hand, diminish the risk for false positive signals. Basically, the same criteria applies to covariates as the quantitative traits. Also dichotomous variables may be used, however, such as gender. Importantly, the genotype information is not suitable as covariate, and additionally, the covariates should correlate with each other as little as possible (Balding 2006).

2.3.6 Statistical significance

In addition to the generic significance values for genome-wide analyses mentioned above, also study-specific significance threshold can be evaluated. Before the analysis, *simulations* based on the study material characteristics may be used to evaluate the adequate threshold values for significance (Balding 2006, Wilkinson 2009).

After the initial analyses, empiric P-values may be calculated by performing *permutation analysis*. In a similar manner than in the simulations, the study material characteristics are used to evaluate the likelihood of obtained results occurring only by chance. However, the results depend upon the number of permutations, which should at least equal the number of performed independent tests (Balding 2006).

Alternatively, the P-values may be corrected for multiple testing. In the conventional *Bonferroni correction*, the desired significance level is divided by the number of independent tests made to obtain the threshold value for individual signals. However, in the genetic studies of complex disorders the Bonferroni correction is usually considered highly conservative as typically neither the markers nor traits are fully independent of each other (Balding 2006). To overcome this, the equivalent number of independent markers and traits may be evaluated from the analyzed material (Nyholt 2004).

In addition to different correction methods, *replication* of the original results in an independent study material is usually considered adequate proof of true positive signal. On the other hand, in the analysis of complex disorders, negative replication results are usually considered insufficient to determine prior positive signals as false ones due to disorder characteristics mentioned before. Furthermore, no definite agreement exists whether a positive association with the same gene/region, the same haplotype(s), the same marker(s), or the same allele(s) constitutes an acceptable replication. In any case, the most rigorous criterion for replication would require the same allele of the same marker to be associated with the exactly same phenotype as in the original study (Burmeister et al. 2008).

2.3.7 Meta-analysis

In meta-analysis, results from multiple individual studies addressing related hypothesis are combined by modelling common measure(s) with regression analysis. Most importantly, the increased sample size allows more power in the analysis. Overall, the results highlighted in meta-analyses supposedly comprise high significance, although some caveats exist. The so-called publication bias, referring to studies with positive findings getting more easily published than those with negative ones, may affect the repertoire of the source studies. Furthermore, strong individual signals may cover weaker ones in the combined analysis (Normand 1999).

In genetic meta-analyses, the studied chromosomal regions are usually divided into sections called bins. Signals located near the boundaries of the bins are prone to be missed, thus the regions are usually analyzed multiple times with different sets of bins. Additionally, the individual bin ranks are usually weighted by the number of affected subjects in the corresponding study (Wise et al. 1999).

2.4 Genetics of schizophrenia

2.4.1 Overview

Traditionally, the genetic risk for complex disorders has been hypothesized to be based on effects of rare variants with large individual effects, or common variants with small individual effects (Reich et al. 2001). These hypotheses are not necessarily contradictory, however, since the rare variants may increase the disorder risk by themselves, whereas the common variants may primarily reflect the effects of the other causative variant(s) in LD with them (Bodmer et al. 2008). At least some of the common variants may still affect the investigated disorder or trait also directly, however, as described in section 2.2.2 (Chamary et al. 2006).

The current hypothesis for the liability of schizophrenia suggests a role for combination of multiple genetic variants in conjunction with environmental factors. Despite the high heritability of the disorder—much higher than that of, for instance, breast cancer—identification of underlying risk variants has proven tedious. Evidently, due to genetic heterogeneity, the risk variant combinations may differ substantially between affected individuals, especially between families not sharing the same ancestry. On the other hand, different phenotypes may result from the same variants, depending on the amount and/or combinations of the variants. Probably only very few patients share identical genetic liability, complicating especially the personalized treatment efforts (Harrison et al. 2005, Rapoport et al. 2005, Burmeister et al. 2008, Keshavan et al. 2008).

2.4.2 Chromosomal abnormalities

A deletion in chromosome 22q11.2 causes Velocardiofacial syndrome (VCFS). This disorder is characterized by congenital dysmorphology, heart disease, and learning disabilities. Additionally, even 24% of these patients fulfill the criteria for schizophrenia (Murphy et al. 1999). On the other hand, only about 0.65% of all schizophrenia patients have deletion(s) in the 22q11 region (Ivanov et al. 2003). These deletions are, however, substantially more prevalent, up to 5.3%, among patients with childhood onset of schizophrenia (Sporn et al. 2004).

One of the best-known examples of familial liability in psychiatric disorders is a large multi-generational Scottish pedigree with a highly elevated risk for major psychiatric disorders, including schizophrenia. This results from an inherited balanced translocation between chromosomes 1q and 11q, t(1;11)(q42;q14.3) (St Clair et al. 1990), which disrupts three genes in chromosome 1q (Millar et al. 2000a, Millar et al. 2000b).

In addition to these, several rare chromosomal aberrations are associated with the schizophrenia liability. Although their significance seems to be only minor at the population level (MacIntyre et al. 2003), at least some of the genes disrupted in these aberrations seem to affect the disorder liability in their functional forms as well, as seen, for instance, for the *Disrupted in schizophrenia 1* (*DISC1*) gene on the aforementioned chromosome 1q translocation region (Hennah et al. 2006).

2.4.3 Copy number variations

Recent progress in large-scale genotyping technologies has enabled systematic screening of CNVs which, in contrast to chromosomal abnormalities, are typically smaller in size and higher in number. Especially deletions larger than one million nucleotides in length, that is, over 1 Mb, are more prevalent among patients with schizophrenia. The actual underlying genetic mechanisms remain somewhat unclear, however. The locations of the CNVs vary, not all of them disrupting known genes, and in addition, the findings are inconsistent concerning the possibly affected pathways involved in, for instance, neuronal development and transmission. Although these variants evidently elevate the disorder risk at the individual level, their impact seems to remain low at the population level (International Schizophrenia Consortium 2008, Stefansson et al. 2008, Walsh et al. 2008, Kirov et al. 2009, Need et al. 2009).

2.4.4 Linked chromosomal regions

Several individual genome-wide linkage studies for schizophrenia have pointed to almost all chromosomal regions (Sullivan 2005). The wide dispersion of the linked regions may result from, for instance, differences in both the utilized samples and their ascertainment, as well as from dissimilarities in the used methodology, including genotyping and analysis techniques. However, it may also reflect the extent of the disorder-related genetic variants. In any case, meta-analyses have highlighted some chromosomal regions harboring plausible candidate genes, and overall supported these regions in the genetic background of schizophrenia (Lewis et al. 2003, Ng et al. 2009) (Table 5). Although the present large-scale genotyping technologies have enabled genome-wide association analysis methods, the linkage studies may still provide some additional information on the candidate chromosomal regions.

In Finland, our genome-wide linkage analyses have revealed schizophrenia susceptibility loci on chromosomes 1q32.2-q42, 2q, 4q, 5q, and 7q22 (Hovatta et al. 1999, Ekelund et al. 2000, Ekelund et al. 2001, Paunio et al. 2001), as well as a locus for visual working memory on chromosome 2q, and a locus for verbal learning and memory on 4q (Paunio et al. 2004). Among others, these cognitive traits showing linkage are considered valid schizophrenia endophenotypes (Gur et al. 2007). Importantly, the 2q and 5q schizophrenia loci have also been highlighted in international schizophrenia linkage meta-analyses (Lewis et al. 2003, Ng et al. 2009). Of these, only the chromosome 1q locus has previously been studied in an extensive manner in the Finnish samples (Hennah et al. 2003, Hennah et al. 2005).

Table 5. Chromosomal regions highlighted in meta-analyses of partly overlapping 20 (Lewis et al. 2003) and 32 (Ng et al. 2009) genome-wide linkage studies of schizophrenia. Only regions with P-value <0.05 are shown. The regions highlighted in both studies are shown in bold. The subset of European studies from (Ng et al. 2009) are shown in italics.

Location	cM[a]	Weighted analysis			Both	Sample
		P_{AR}[b]	P_{SR}[c]	P_{OR}[d]	P<0.05	
1p32.2-p31.1	85.8-114.5	NA	0.02692	0.08449		ALL (32)
1p13.3-q23.3	**142.2-170.8**	**0.0235**	**NA**	**0.0136**	*	**ALL (20)**
1p13.2-q23.3	**143.1-171.7**	**NA**	**0.00814**	**0.06639**		**ALL (32)**
1q23.3-q31.1	170.8-201.6	0.082	NA	0.0142		ALL (20)
2p12-q22.1	**101.6-128.4**	**0.0004**	**NA**	**0.0327**	*	**ALL (20)**
2q12.1-q21.2	**117.5-146.9**	**NA**	**0.00755**	**0.22798**		**ALL (32)**
2q22.1-q23.3	**128.4-154.5**	**0.023**	**NA**	**0.0448**	*	**ALL (20)**
2q21.2-q31.1	**146.9-176.3**	**NA**	**0.02395**	**0.14269**		**ALL (32)**
2q33.3-q36.3	205.7-235.1	NA	0.00916	0.0197	*	ALL (32)
		NA	*0.01016*	*0.35077*		*EUR (22)*
3p25.3-p22.1	32.4-63.1	0.006	NA	0.0311	*	ALL (20)
3p14.1-q13.32	95.9-127.9	NA	0.04047	0.0114	*	ALL (32)
		NA	*0.04359*	*0.62724*		*EUR (22)*
5q23.2-q34	**131.5-164.2**	**0.0032**	**NA**	**0.0491**	*	**ALL (20)**
5q31.3-q35.1	**148.9-178.7**	**NA**	**0.00459**	**0.43156**		**ALL (32)**
		NA	***0.01718***	***0.33717***		***EUR (22)***
5q35.1-q35.3	178.7-208.5	NA	0.0276	0.0343	*	ALL (32)
6pter-p22.3	0-32.6	0.0159	NA	0.0328	*	ALL (20)
6p22.3-p21.1	**32.6-65.1**	**0.033**	**NA**	**0.0024**	*	**ALL (20)**
6p21.31-p12.1	***56.0-84.0***	***NA***	***0.04433***	***0.44626***		***EUR (22)***
6q15-q23.2	99.0-131.1	0.098	NA	0.0177		ALL (20)
8p22-p21.1	**27.4-55.0**	**0.031**	**NA**	**0.0068**	*	**ALL (20)**
8p22-p12	**28.1-56.2**	**NA**	**0.03086**	**0.0207**	*	**ALL (32)**
		NA	***0.00057***	***0.06659***		***EUR (22)***
10pter-p14	0-29.2	0.068	NA	0.0046		ALL (20)
10q26.12-q26.3	145.9-175.0	NA	0.03487	0.0135	*	ALL (32)
11q22.3-q24.1	99.0-123.0	0.006	NA	0.004	*	ALL (20)
14pter-q13.1	0-40.1	0.047	NA	0.0043	*	ALL (20)
15q21.3-q26.1	52.3-85.6	0.095	NA	0.0293		ALL (20)
16p13-q12.2	**32.1-67.6**	**0.056**	**NA**	**0.0069**		**ALL (20)**
16p13.12-q12.2	***33.3-66.7***	***NA***	***0.01775***	***0.15808***		***EUR (22)***
17q21.33-q24.3	63.6-94.0	0.112	NA	0.0349		ALL (20)
18q22.1-qter	96.5-126.0	0.065	NA	0.0103		ALL (20)
20p12.3-p11	21.2-47.5	0.046	NA	0.0098	*	ALL (20)
22pter-q12.3	0-33.8	0.031	NA	0.0216	*	ALL (20)

[a]Marshfield map position (http://research.marshfieldclinic.org/genetics/) from (Lewis et al. 2003), Rutgers map position (Matise et al. 2007) from (Ng et al. 2009), both presented as centiMorgans (cM)
[b]P-value for average rank (AR) analysis (bin ranks averaged across all studies) (Lewis et al. 2003)
[c]P-value for summed rank (SR) analysis (bin ranks summed across all studies) (Ng et al. 2009)
[d]P-value for ordered rank (OR) analysis (bin ranks ordered according to their values and their places in the order taken into account) (Lewis et al. 2003, Ng et al. 2009)
Abbreviations: ALL (20), all 20 studies in (Lewis et al. 2003); ALL (32), all 32 studies in (Ng et al. 2009); EUR (22), subset of 22 European studies in (Ng et al. 2009); NA, not assessed

2.4.5 Candidate genes

The linkage studies, detected structural variations, and the known gene functions have traditionally set the basis for candidate gene selection for association studies. For the development in technology and methodology, the focus in association analyses is already shifting towards the whole genome association analyses (Burmeister et al. 2008). In recent large-scale genome-wide association studies, the only consistent finding has been the major histocompatibility complex (MHC) region on chromosome 6p22 (International Schizophrenia Consortium et al. 2009, Shi et al. 2009, Stefansson et al. 2009), harboring several genes playing important roles in the immune system and autoimmunity (The MHC sequencing consortium 1999). Overall, the studies have supported the hypothesis of the genetic liability to schizophrenia involving several common variants with small individual effects, and additionally, that the major psychiatric disorders share at least some factors in their genetic background which, on the other hand, are not shared with non-psychiatric disorders (O'Donovan et al. 2008, International Schizophrenia Consortium et al. 2009, Shi et al. 2009, Stefansson et al. 2009).

At present, no single major genetic determinant has been identified, although several genes have been highlighted across the genome (Table 6). That the variants showing association have often varied within the genes may stem from differences in the LD structures of the samples, assuming that none of the analyzed markers is the actual causative variant (Balding 2006). It may, however, also imply underlying allelic heterogeneity, that is, multiple variants in the same gene affecting the liability (Burmeister et al. 2008). Of the strongest candidate genes, some relevant are described in detail below.

The balanced t(1:11)(q43,q21) translocation inherited in one Scottish pedigree disrupts the *Disrupted in schizophrenia 1* (*DISC1*) [OMIM 605210], *Disrupted in schizophrenia 2* (*DISC2*) [OMIM 606271], and *Translin-associated factor X* (*TSNAX*) [OMIM 602964] genes on chromosome 1q42.1-q42.2 (Millar et al. 2000a, Millar et al. 2000b). Numerous members of the pedigree show symptoms fulfilling criteria for several major mental illnesses, including schizophrenia (St Clair et al. 1990). Interestingly, the linkage signal to the translocation region increases when the disorder phenotype is broadened from schizophrenia to include also other major affective disorders (Blackwood et al. 2001), supporting the view of their overall overlapping genetic liability (Kendler et al. 1993a, Kendler et al. 1993b, Kendler et al. 1993c, Kendler et al. 1993d, Tienari et al. 2003). In the brain, *DISC1* is widely expressed and participates in regulation of neuronal migration and intercellular transport (Miyoshi et al. 2003, Ozeki et al. 2003). In mice, *Disc1* missense mutation causes physiological and phenotypic changes similar to schizophrenia and major depression (Clapcote et al. 2007). Interestingly, antipsychotic medication reverses these features to some degree (Clapcote et al. 2007). In Finland, human *DISC1* markers and/or haplotypes have shown association with schizophrenia, (Hennah et al. 2003), bipolar disorder [OMIM 125480] (Palo et al. 2007), autism [OMIM 209850] (Kilpinen et al. 2008), and Asperger syndrome [OMIM 608638] (Kilpinen et al. 2008). Furthermore, *DISC1* may affect cognitive functioning, including visual memory (Hennah et al. 2005) and verbal memory (Cannon et al. 2005). Interestingly, *DISC1* variants show association with social anhedonia, a psychosis-related trait, in general population (Tomppo et al. 2009). Due to the wide spectrum of evidence, *DISC1* is considered perhaps the most promising candidate gene for schizophrenia liability.

The *Dystrobrevin binding protein 1* (*DTNBP1*) [OMIM 607145] gene on chromosome 6p22.3 encodes for a neuronal protein expressed widely in the human brain (Weickert et al. 2004). *DTNBP1* locates in schizophrenia-linked region (Straub et al. 1995), is associated with schizophrenia (Straub et al. 2002) (Figure 4), as well as cognitive functions (Donohoe et al. 2007), and its expression is reduced in schizophrenia (Weickert et al. 2004, Weickert et al. 2008). Although the specific function of *DTNBP1* remains unknown, and the identified risk variants have varied between the several studies revealing associations with schizophrenia (Figure 8), *DTNBP1* is considered one of the most promising candidates for the liability.

The *V-akt murine thymoma viral oncogene homolog 1* (*AKT1*) [OMIM 164730] gene on chromosome 14q32.32-q32.33 encodes for a serine/threonine kinase which is widely expressed in the brain and participates in neuronal proliferation and maintenance (Wang et al. 2003). The AKT pathway is associated with schizophrenia liability (Emamian et al. 2004), and putatively with bipolar disorder (Toyota et al. 2003). Interestingly, overexpression of *DTNBP1* elevates the AKT activity via phosphorylation, and thus increases its neuronal protective properties, and vice versa (Numakawa et al. 2004). In rats, the Akt pathway is associated with spatial memory functions (Mizuno et al. 2003). In humans, *AKT1* is also associated with verbal learning and memory, as well as cortical gray matter density (Pietilainen et al. 2009).

The *Glutamate receptor, metabotropic 3* (*GRM3*) [OMIM 601115] gene on chromosome 7q21.1-q21.2 participates in glutamatergic neurotransmission (Cartmell et al. 2000). In rats, both Grm2 and Grm3 agonists reverse the effects of phencyclidine (PCP), a drug which induces schizophrenia-like symptoms (Moghaddam et al. 1998). Furthermore, chronic exposure to olanzapine, an antipsychotic drug, upregulates Grm2 and Grm3 (Tascedda et al. 2001). In humans, *GRM3* has shown association with schizophrenia (Fujii et al. 2003, Egan et al. 2004, Chen et al. 2005), and cognitive functions, including verbal learning (Egan et al. 2004).

The *Semaphorin 3A* (*SEMA3A*) [OMIM 603961] gene on chromosome 7q21.11 is involved in axon guidance, and regulates dendritic attraction, possessing primarily inhibitory functions (He et al. 2002). In human brain, *SEMA3A* expression is increased in schizophrenia (Eastwood et al. 2003). However, any direct genetic association with schizophrenia remains undetermined.

The *Reelin* (*RELN*) [OMIM 600514] gene on chromosome 7q22 encodes for a glycoprotein involved in neuronal migration regulation and synaptic plasticity (D'Arcangelo et al. 1995, DeSilva et al. 1997, Rice et al. 2001, Fatemi 2005). Functionally, the most important regions of Reelin are the conserved C-terminus, crucial for downstream signaling (Nakano et al. 2007), and reelin repeats five and six out of the total eight, forming the essential binding site (Yasui et al. 2007) for the Very-low-density lipoprotein receptor (VLDLR), Apolipoprotein E receptor 2 (ApoER2), and Alpha-3-beta-1 integrin receptor (α3β1) (D'Arcangelo et al. 1999, Dulabon et al. 2000). In mice, the *reeler* phenotype of *Reln* null mice involves malformation of the brain cortex and severe phenotypic changes, such as tremor and ataxia (D'Arcangelo et al. 1998). In addition, heterozygous *Reln* null mutation as well as homozygous null mutation of Reelin receptor *Apoer2* or *Vldlr* result in cognitive disturbances (Qiu et al. 2006, Barr et al. 2007). In human brain, reduced Reelin protein and/or mRNA levels are detected in schizophrenia (Impagnatiello et al. 1998), autism (Fatemi et al. 2005), lissencephaly [OMIM 257320] (Hong et al. 2000), bipolar disorder (Guidotti et al. 2000)

and major depression [OMIM 608516] (Fatemi et al. 2000). *RELN* expression is reduced by its promoter-region hypermethylation (Chen et al. 2002), and in post-mortem brain studies, *RELN* promoter regions important for transcriptional regulation have usually been hypermethylated in schizophrenia patients (Grayson et al. 2005). *RELN* methylation may, however, increase with age as well (Tamura et al. 2007). *RELN* expression is also reduced by its promoter-region trinucleotide repeat lengthening (Persico et al. 2006) which in turn is associated with treatment-resistant schizophrenia (Goldberger et al. 2005). In addition to its promoter-region, however, only a few direct genetic associations between schizophrenia and *RELN* exist (Kahler et al. 2008, Shifman et al. 2008), thus leaving the specific mechanisms underlying the disorder liability somewhat unclear.

The *Neuregulin 1* (*NRG1*) [OMIM 142445] gene on chromosome 8p21-p12 encodes for a growth factor with multiple splice variants, several of which possess extensive roles in both the development and functioning of the brain, including modulation of neuronal migration, synaptogenesis, and neurotransmission (Harrison et al. 2006). Like *DTNBP1*, also *NRG1* locates in schizophrenia-linked region (Pulver et al. 1995), and has been associated with schizophrenia (Stefansson et al. 2002), although the identified risk variants differ between the several studies showing association (Figure 8). At present, *NRG1* is considered one of the most promising genes in schizophrenia liability.

The *V-erb-a erythroblastic leukemia viral oncogene homolog 4* (*ERBB4*) [OMIM 600543] gene on chromosome 2q34 encodes for a *NRG1* receptor. *ERBB4* shows association with schizophrenia both in candidate gene (Norton et al. 2006, Silberberg et al. 2006) and genome-wide studies (Shi et al. 2009). A possible underlying mechanism is altered *ERBB4* splicing in schizophrenia (Law et al. 2007). Furthermore, copy number variations in *ERBB4* are overrepresented among patients with schizophrenia (Walsh et al. 2008). Altered *NRG1-ERBB4* signaling is likely to contribute to *N-methyl D-aspartate glutamate receptor* (*GRIN*; formerly *NMDAR*) hypofunction in schizophrenia (Hahn et al. 2006). In mice, *Erbb4* null mutation shows phenotypic similarities with that of schizophrenia (Golub et al. 2004). Also in healthy individuals, *ERBB4* may influence cognitive functioning, as seen for verbal working memory (Nicodemus et al. 2006).

Table 6. The schizophrenia candidate genes considered most promising in four recent reviews (Owen et al. 2004, Harrison et al. 2005, Rapoport et al. 2005, Keshavan et al. 2008) and/or highlighted in the Schizophrenia Research Forum (http://www.schizophreniaforum.org/) as in July 2009.

Gene	OMIM	Locus	Function	In schizophrenia				
				BP[a]	GA[b]	AE[c]	AS[d]	CNV[e]
MTHFR	607093	1p36.3	Homocysteine metabolism; enzyme	+	+++	NK	(+)	NK
RGS4	602516	1q23.3	G protein signaling; regulator	++	+++	-	(+)	NK
PLXNA2	601054	1q32.2	CNS development; semaphorin receptor	+++	++	NK	NK	NK
DISC1	605210	1q42.1	CNS development and neural plasticity; regulator	+++	+++	NK	NK	NK
IL1B	147720	2q14	Inflammatory response; cytokine	++	++	NK	NK	NK
GAD1	605363	2q31	Glutamate metabolism; enzyme	+++	+++	-	NK	NK
DLX1	600029	2q31.1	CNS development; transcription factor	++	+	-	NK	NK
ZNF804A	612282	2q32.1	Zinc finger protein	0	+	NK	NK	NK
ERBB4	600543	2q33.3-q34	Neuregulin receptor	+++	+++	+	+	+
DRD3	126451	3q13.3	Dopamine receptor	+++	+++	(+)	+	NK
GABRB2	600232	5q34	Inhibitory synaptic transmission; GABA A receptor	+++	+++	(+/-)	+	NK
DRD1	126449	5q34-q35	Dopamine receptor	+++	0	NK	NK	NK
DTNBP1	607145	6p22.3	Component of dystrophin-associated protein complex	+	+++	-	NK	NK
SEMA3A	603961	7q21.11	CNS development; inhibitory regulator	+++	0	+	NK	NK
GRM3	601115	7q21.1-q21.2	Glutamate receptor	+++	+++	0	(+)	NK
RELN	600514	7q22	CNS development and neural plasticity; stimulatory regulator	+++	++	-	NK	NK
PPP3CC	114107	8p21.3	Phosphorylation; enzyme	+++	+	(-)	NK	NK
SLC18A1	193002	8p21.3	Neurotransmitter transporter (synaptic vesicles)	++	++	NK	(+)	NK
NRG1	142445	8p21-p12	CNS development; stimulatory regulator	+++	+++	(+)	NK	NK
NA	NA	10q26.13	Not known	NA	+	NA	NA	NA
DRD4	126452	11p15.5	Dopamine receptor	+++	+++	NK	NK	+
TPH1	191060	11p15.3-	Serotonin	+++	+++	(+)	NK	NK

		p14	biosynthesis; enzyme					
NA	NA	11p14.1	Not known	NA	+	NA	NA	NA
DRD2	126450	11q22-q23	Dopamine receptor	+++	+++	(+/-)	(+/-)	+
OPCML	600632	11q25	Immunoglobulin	0	+	NK	NK	NK
GRIN2B	138252	12p12	Glutamate receptor	+++	+++	NK	NK	NK
DAO	124050	12q24	Glutamate metabolism; enzyme	+++	++	(+)	+	NK
HTR2A	182135	13q14-q21	Serotonin receptor	+++	+++	NK	NK	NK
DAOA	607408	13q33.2	DAO activator	+	+++	NK	NK	NK
AKT1	164730	14q32.32-q32.33	CNS development; neuron survival regulator	+	+++	-	NK	NK
CHRNA7	118511	15q13.3	Cholinergic receptor	++	+++	-	NK	NK
NA	NA	16p13.12	Not known	0	+	NA	NA	NA
RPGRIP1L	610937	16q12.2	Cilia and centrosome function	++	+	NK	NK	+
HP	140100	16q22.1	Hemoglobin binding	++	+++	+	NK	NK
TP53	191170	17p13.1	Cell cycle regulation; transcription factor	++	++	NK	NK	NK
APOE	107741	19q13.31	Lipoprotein metabolism; transporter	+++	+++	+	+	NK
PRODH	606810	22q11.2	Proline metabolism; enzyme	+	+++	NK	NK	+
DGCR2	600594	22q11.21	Neuronal migration; adhesive receptor	+++	++	+	NK	+
COMT	116790	22q11.21	Catecholamine neurotransmitter inactivation; enzyme	+++	+++	(+/-)	NK	NK

[a]Biological plausibility: 0, none; +, weak; ++, suggestive; +++, strong
[b]Genetic association: 0, none; +, single; ++, some (≤3); +++, multiple (>3)
[c]Altered expression: 0, none; -, decreased; +, increased; (), uncertain
[d]Alternative splicing: +, some variant(s) overrepresented; (), uncertain
[e]Copy number variations: +, yes
Abbreviations: OMIM, Online Mendelian Inheritance in Man; NA, not available; NK, not known; for gene name abbreviations, refer to the Abbreviations chapter

3 Aims of the study

The aim of this study was to investigate the genetic background of schizophrenia in the Finnish nationwide sample of familial schizophrenia, collected by the National Institute for Health and Welfare (formerly National Public Health Institute). Specifically, the aims were:

1. To characterize the impact of common variants in the schizophrenia candidate genes *DTNBP1*, *NRG1*, and *AKT1* in Finnish schizophrenia families (I).

2. To replicate the schizophrenia linkage on chromosome 7q22 highlighted in our previous study (Ekelund et al. 2000), and to further examine the regional candidate genes in an extended sample of Finnish schizophrenia families (II).

3. To replicate the detected association between the *RELN* gene and cognitive functions impaired in schizophrenia, and to investigate further whether *RELN* variants modify the clinical features of schizophrenia, or are associated with cognitive disturbances in an extended sample of Finnish schizophrenia families and controls from general population (III).

4. To survey altogether 104 candidate genes on chromosomal regions 2q33.1-2q37.3, 4q13.1-4q26, and 5q31.1-5q33.3, highlighted in our previous linkage analyses (Paunio et al. 2001, Paunio et al. 2004), by utilizing both diagnostic information and cognitive test measurements in Finnish schizophrenia families and controls from general population (IV).

4 Ethical considerations

This study has followed the Declaration of Helsinki (World Health Organization, 1964) and its amendments in full. The research plan has been approved by the Ministry of Social Affairs and Health, Finland (Dnro 105/07/98, 29.4.1999), the Ethics Committees of National Institute for Health and Welfare (6093, 7.10.1998) and the Hospital District of Helsinki and Uusimaa, Finland (HUS 434/E0/05, 3.1.2006), and by the appropriate institutional review boards of the participating institutions. Written informed consent has been obtained from all the participants.

The confidentiality of the participants has been assured by using anonymous numeric coding in all the analyses and data handling. Only specified senior researchers have been allowed to access the information behind the coding and the personal identifying information.

5 Materials and methods

5.1 Study samples

5.1.1 Schizophrenia family sample

The utilized schizophrenia family samples were nested within the Finnish schizophrenia family study sample of the National Institute for Health and Welfare (formerly National Public Health Institute) (I-IV). This series has been collected by using three nationwide medical registers (Hospital Discharge Register, and Pension and Reimbursement Registers) and the Population Register Centre for pedigree information. In this series, the nuclear families have been collected both from an internal isolate (IS) from northeastern Finland with a higher lifetime morbid risk (3.2%) of schizophrenia (Hovatta et al. 1997), and from the general population from rest of Finland (AF; all Finland) with a similar morbid risk (1.1%) of schizophrenia (Perala et al. 2007) than elsewhere (Saha et al. 2005) (Figure 4b). The contacted families from the isolate involved at least one affected individual, whereas the contacted families originating outside the isolate comprised at least two affected individuals. Although the affected individuals in the participating families have had more hospital treatments than the refused ones, no significant clinical differences exist between these groups (Juvonen et al. 2000).

In these families, at least one affected individual was born between 1940 and 1976, with the first schizophrenia diagnosis in any of the registers made between 1969 and 1998. Clinical data were collected from all the mental health treatment contacts, and final diagnostic assessments was made independently by two, or three if necessary to gain consensus, psychiatrists or psychiatric residents according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria (American Psychiatric Association 1994). One of the psychiatrists also completed the Operational Criteria Checklist (OPCRIT) (McGuffin et al. 1991) (Figure 6).

In this series, altogether 983 affected and unaffected individuals have been interviewed with the Structured Clinical Interview for DSM-IV (SCID-I) (First et al. 1997) and have been tested with a comprehensive neuropsychological test battery (Tuulio-Henriksson et al. 2002). These tests cover the central cognitive functions impaired in schizophrenia (Heinrichs et al. 1998). Additionally, the interview included the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen 1984) for the affected individuals, and the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen 1983) for the affected individuals as well as their healthy family members (II-IV) (Figure 6).

This family sample was also divided into two genealogical subcategories, families from the internal isolate (IS), and families outside the isolate (AF; all Finland) (Figure 4b). This further division of the families is justified by both the disorder status distribution in Finland (Hovatta et al. 1997), and the known genetic substructure of the Finns (Salmela et al. 2008, Jakkula et al. 2008). In study I, the inclusion criterion for families from the isolate was based on at least one parent born in the geographical isolate region, the criterion used previously in our genome-wide linkage analyses (Paunio et al. 2001). In study II, the genealogical information allowing (Hovatta et al. 1999, Varilo et al. 2000), the inclusion criterion was tightened, and was based on at least one maternal and one

paternal grandparent born in the geographical isolate region. For studies III and IV, novel identical by state (IBS) clustering information was available from families with a member genotyped with Illumina HumanHap300 platform (Illumina inc., San Diego, CA, USA) as part of the SGENE consortium (http://www.sgene.eu/), and thus, the inclusion criterion was based primarily on this clustering information, and secondarily, on at least one maternal and one paternal grandparent born in the geographical isolate region. In all, roughly 20% of the families were differentially categorized depending on the use of the IBS clustering information.

In Study I, the whole sample comprised altogether 1864 individuals belonging to 441 nuclear families (171 IS and 270 AF families). Of these, altogether 638 individuals were affected with schizophrenia (liability class 1; LC1), and 865 individuals were affected with schizophrenia spectrum disorders, including schizophrenia (liability class 3; LC3). The samples used in Study II, III, and IV are detailed in Table 7 and Table 8.

Figure 6. Identification and collection of the schizophrenia family sample.

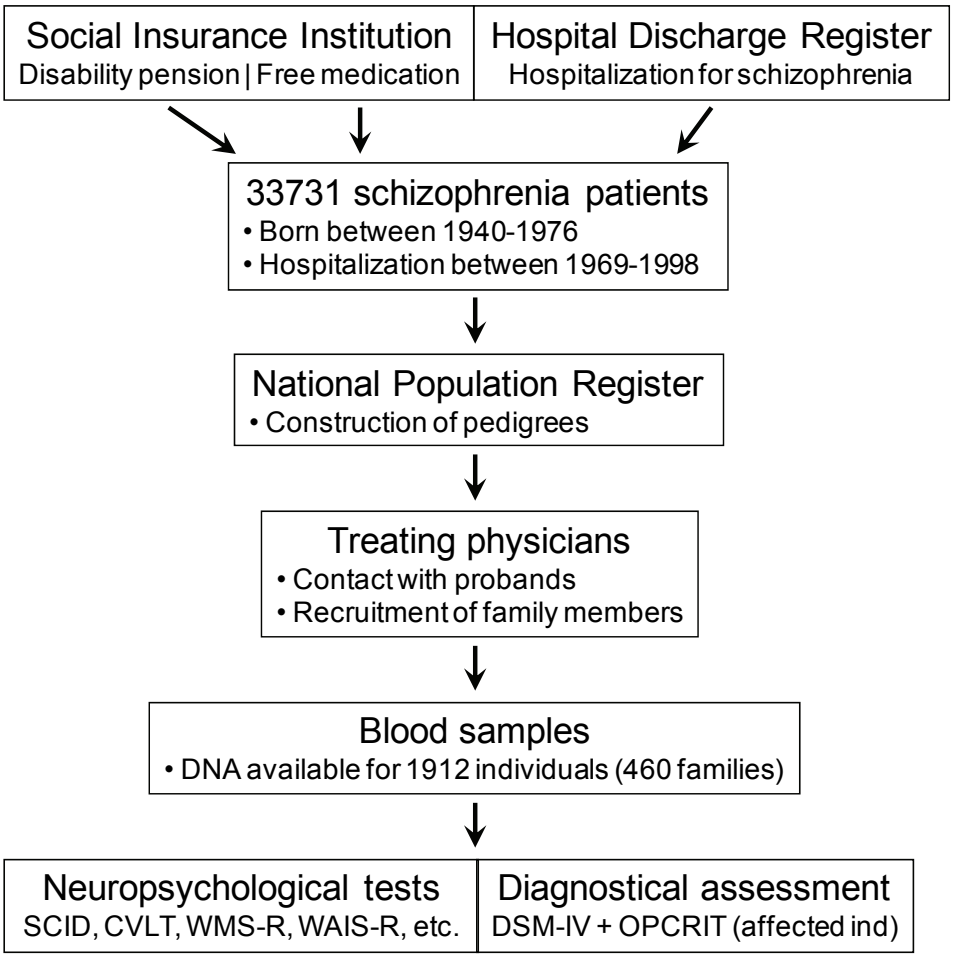


Table 7. Sample in study II. a) The replication linkage analysis sample excludes the original 52 families demonstrating linkage to chromosome 7q22 (Ekelund et al. 2000). b) The qualitative trait association analysis sample is nested within families originating from the AF region in the replication and the original linkage analysis samples (Ekelund et al. 2000). c) The quantitative trait analysis sample is nested within the total replication and the original linkage analysis samples (Ekelund et al. 2000).

	Fam	**Ind**	**Affected**			**Add phen data**
			LC1	**LC2**	**LC3**	**NP**
a) Linkage analysis of clinical diagnostic categories						
Total	352	1626	480	586	657	
Males		825	302	357	398	
Females		801	178	229	259	
AF	256	1211	387	477	541	
Males		608	245	291	326	
Females		603	142	186	215	
IS	96	415	93	109	116	
Males		217	57	66	72	
Females		198	36	43	44	
b) Candidate gene analysis of clinical diagnostic categories						
Total (AF)	245	1074	369	442	503	
Males		528	227	260	289	
Females		546	142	182	214	
c) Candidate gene analysis of neuropsychological test measurements						
Total	186	861	234	274	303	618
Males			119	138	150	324
Females			115	136	153	294
AF	93	451	143	165	187	326
Males			72	84	92	175
Females			71	81	95	151
IS	93	410	91	109	116	292
Males			47	54	58	149
Females			44	55	58	143

Abbreviations: Fam, families; Ind, individuals; Add phen data, additional phenotype data; LC1, liability class 1 (schizophrenia); LC2, liability class 2 (LC1 + schizoaffective disorder); LC3, liability class 3 (LC2 + other schizophrenia spectrum disorders); NP, neuropsychological tests; IS, internal isolate; AF, all Finland (outside the isolate)

Table 8. Samples in a) study III and b) study IV. In both studies, the samples comprise all the neuropsychologically assessed families available in the total schizophrenia study sample. The differences in amount of subjects are due to divergent genotyping technologies utilized in the studies.

	Fam	Ind	Affected			Additional phenotype data			
			CORE-SCH	SCH-SPECT	ANY-PSY	NP	SAPS	SANS	OPCRIT
a) Sample in study III									
Total	290	1259	297	409	464	892	476	709	474
Males		640	191	250	271	457	274	381	282
Females		619	106	159	193	435	202	328	192
AF	109	518	135	189	210	367	192	259	214
Males		268	83	109	119	185	107	139	125
Females		250	52	80	91	182	85	120	89
IS	181	741	162	220	254	525	284	450	260
Males		372	108	141	152	272	167	242	157
Females		369	54	79	102	253	117	208	103
b) Sample in study IV									
Total	293	1111	283	402	454	877	463	692	461
Males		573	182	246	265	448	265	369	273
Females		538	101	156	189	429	198	323	188
AF	110	454	127	182	202	355	182	248	203
Males		236	79	105	114	178	100	132	117
Females		218	48	77	88	177	82	116	86
IS	183	657	156	220	252	522	281	444	258
Males		337	103	141	151	270	165	237	156
Females		320	53	79	101	252	116	207	102

Abbreviations: Fam, families; Ind, individuals; CORESCH, schizophrenia; SCHSPECT, CORESCH + other schizophrenia spectrum psychotic disorders; ANYPSY, SCHSPECT + major affective disorders with psychotic features; NP, neuropsychological tests; SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the assessment of Negative Symptoms; OPCRIT, Operational Criteria Checklist; IS, internal isolate; AF, all Finland (outside the isolate)

5.1.2 Control samples

In study II, the parents (n=114) from altogether 57 anonymous trios (father, mother, and child) were used to define the haplotype blocks and allelic variation in the general Finnish population. These parents with unknown phenotypes were also included in association analyses as independent healthy controls. Here, the risk for including some affected individual(s) wrongly as unaffected is relatively low, however, since the lifetime risk of schizophrenia is smaller in samples with known parenthood than in randomly selected samples of unrelated individuals (Gottesman et al. 1982, Kendler et al. 1993a).

In studies III and IV, the control sample was nested within the nationwide Health 2000 survey (http://www.terveys2000.fi/) of altogether 9922 Finnish adults from 80 municipalities, and comprised altogether 375 unrelated individuals (180 males, 195 females). Of these, 205 were chosen from the Psychoses in Finland (PIF) (Perala et al. 2007) and 82 from the Mental Health in Early Adulthood in Finland (Suvisaari et al. 2009) subsamples of the Health 2000 survey based on available neuropsychological test data and negative history for any psychotic disorder. The remaining 88 individuals, of which 43 were neuropsychologically assessed by using the same protocol as in the PIF study (Perala et al. 2007), were selected for regional controls living in or near the internal isolate (IS) (Hovatta et al. 1997). Of the altogether 330 neuropsychologically tested individuals, 150 were males and 180 females. The control sample was not further divided into genealogical subcategories due to the lack of family history information comparable with the schizophrenia family sample.

5.2 Test variables

5.2.1 Qualitative traits

The used diagnostic classes varied slightly between the studies. In study II, the utilized liability classes (LC) were LC1 consisting of schizophrenia only, LC2 adding schizoaffective disorder, and LC3 adding other schizophrenia spectrum disorders (schizophreniform, delusional and brief psychotic disorder, and schizoid, schizotypal and paranoid personality disorder), and psychotic disorder not otherwise specified. Of these, LC1 and LC3 were utilized in study I. For studies III and IV, the diagnostic classes were reconstructed, and the utilized were CORESCH consisting of schizophrenia only, SCHSPECT adding other schizophrenia spectrum psychotic disorders (schizoaffective, schizophreniform, delusional, brief psychotic, psychotic, and shared psychotic disorders), and ANYPSY adding both bipolar type I or II disorders with psychotic features, and major depressive disorder with psychotic features. Thus, the classes LC1 and CORESCH are equal, and LC3 and SCHSPECT are highly equal (Table 7, Table 8).

Additionally, in study IV, poor premorbid work and social adjustments, manic-type mood symptoms (either elevated or irritable mood), and widespread delusions were examined as clinical features of schizophrenia based on the OPCRIT ratings (McGuffin et al. 1991) (Table 8).

5.2.2 Quantitative traits

In the schizophrenia (II-IV) and control samples (III, IV), the selected test variables from the Wechsler Memory Scale-Revised (WMS-R) (Wechsler 1987), the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler 1981), the California Verbal Learning Test (CVLT) (Delis et al. 1987), the Trail Making Test (TMT) (Reitan et al. 1993), and the Stroop Task Interference Score (Golden 1978) measure variations in attention, working memory, verbal learning and memory, information processing, and executive functioning (Heinrichs et al. 1998) (Table 9). The affected schizophrenia family members showed poorer average level of performance in these traits than their unaffected family members who, in turn, showed poorer performance than the controls from the general population. These characteristics are in line with the endophenotype concept and thus support the use of these traits in the analyses (Gottesman et al. 1973, Gottesman et al. 2003) (Figure 7).

In the schizophrenia sample (III, IV), the age of onset was defined as the earliest age when medical advice was sought for psychiatric reasons, or when symptoms began to cause subjective distress or dysfunction based on the OPCRIT rating (McGuffin et al. 1991). The severity of positive symptoms was assessed with the sum of all the SAPS items (Andreasen 1984), and the severity of negative symptoms with the sum of all the SANS items (Andreasen 1983). Although these symptom ratings are prone to changes according to disorder phase, in the study subject interviews, all available lifelong clinical data were evaluated and taken into account whenever possible (Table 9).

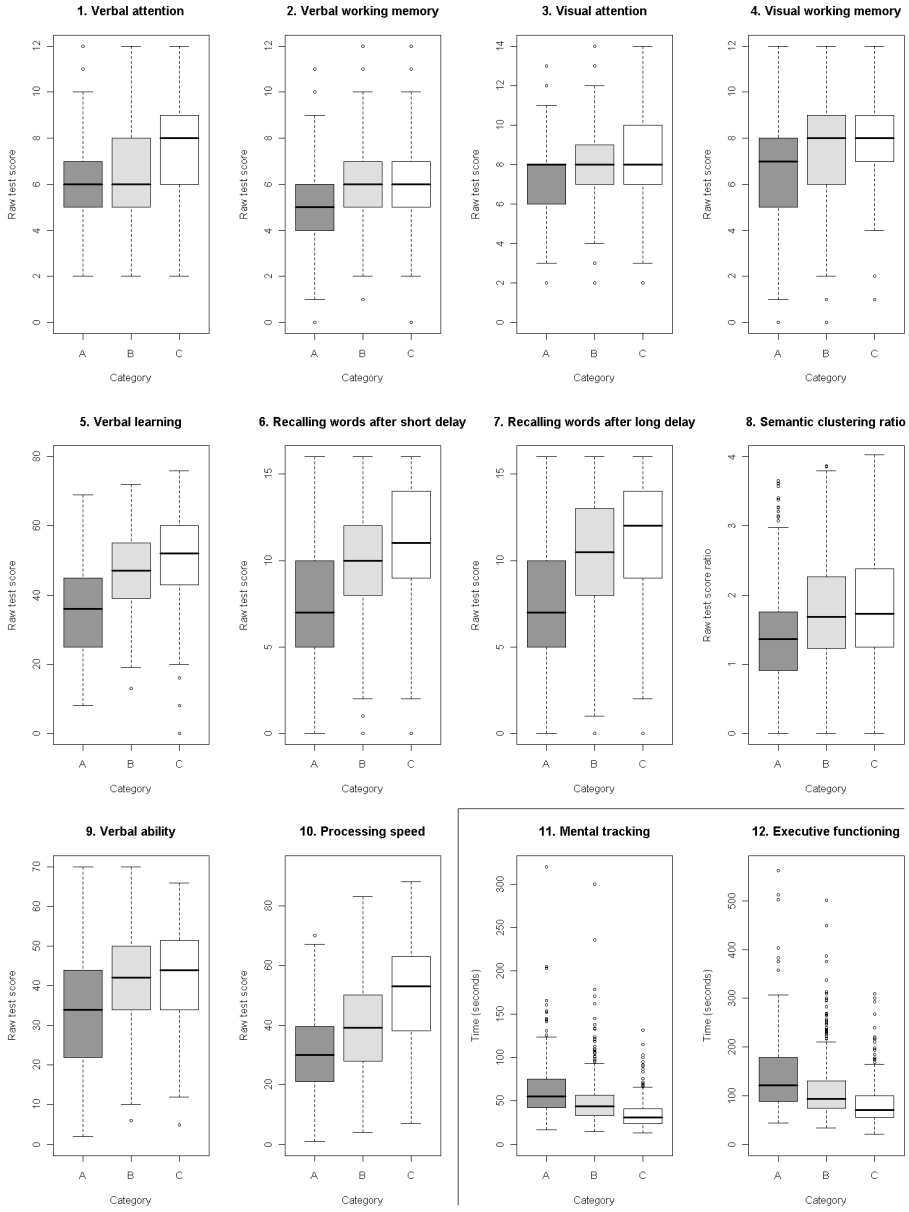
For all the traits, raw scores or transformed raw scores were used in the analyses. The normality of the trait value distributions were verified by using SPSS 16.0.1 (SPSS Inc., Chicago, IL, USA), and the raw scores were transformed if the skewness and/or kurtosis of the trait value distribution was <-1.0 or >1.0.

Table 9. Analyzed quantitative traits. Further information on test scores and their distribution characteristics in the utilized samples are available in the referred original publications.

Trait (abbreviation)	Test	Subtest/definition	Study
Attention and working memory			
Verbal attention (VeAt)	WMS-R	Digit Span forward	II-IV
Verbal working memory (VeWM)	WMS-R	Digit Span backward	II-IV
Visual attention (ViAt)	WMS-R	Visual Span forward	II-IV
Visual working memory (ViWM)	WMS-R	Visual Span backward	II-IV
Mental tracking (MT)	TMT	Trail Making A	III,IV
Verbal learning and memory			
Verbal learning (L)	CVLT	Word list recall in five trials	II-IV
Recalling words after short delay (SDe)	CVLT	Recall after 5 minutes	II-IV
Recalling words after long delay (LDe)	CVLT	Recall after 20 minutes	II-IV
Semantic clustering ratio (SC)	CVLT	Learning strategy	II-IV
Perseverative recall errors (PRE)	CVLT	Repeating words	II
Intrusive recall errors (IRE)	CVLT	Words not in the list	II
Immediate recall of a story (IR)	WMS-R	Logical Memory, immediate	II
Delayed recall of a story (DR)	WMS-R	Logical Memory, delayed	II
Visual learning and memory			
Immediate visual recall (IV)	WMS-R	Visual Reproduction, immediate	II
Delayed visual recall (DV)	WMS-R	Visual Reproduction, delayed	II
Ability functions			
Verbal ability (VeAb)	WAIS-R	Vocabulary	II-IV
Processing speed (PS)	WAIS-R	Digit Symbol	II-IV
Executive functions			
Executive functioning (EF)	TMT	Trail Making B	III,IV
Stroop interference score (S)	Stroop	Stroop task	II-IV
Positive symptoms			
Severity of symptoms (SAPS)	SAPS	All items	III,IV
Negative symptoms			
Severity of symptoms (SANS)	SANS	All items	III,IV
Age of onset			
Earliest age of dysfunction (AOO)	OPCRIT	Item 4	III,IV

Abbreviations: WMS-R, Wechsler Memory Scale-Revised; TMT, Trail Making Test; CVLT, California Verbal Learning Test; WAIS-R, Wechsler Adult Intelligence Scale-Revised; Stroop, Stroop Task Interference Score; SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the assessment of Negative Symptoms; OPCRIT, Operational Criteria Checklist

Figure 7. Neuropsychological test differences (raw test scores) between individuals (n=385) affected with psychosis according to ANYPSY criteria (category A), their unaffected family members (n=536) (category B), and healthy controls (n=330) from general population (category C). For traits 1 to 10, lower test scores, and for traits 11 and 12, higher test scores represent poorer performance. The rectangle ends in the boxplots represent the lower (Q1) and upper (Q3) quartiles, the horizontal line in the rectangles the median value, the whiskers the smallest and largest non-outlier values [lower limit: Q1-1.5*(Q3-Q1)); upper limit: Q3+1.5*(Q3-Q1)], and the circles the outlier values. Modified with permission from Figure S2 in study III (Wedenoja et al. 2009).



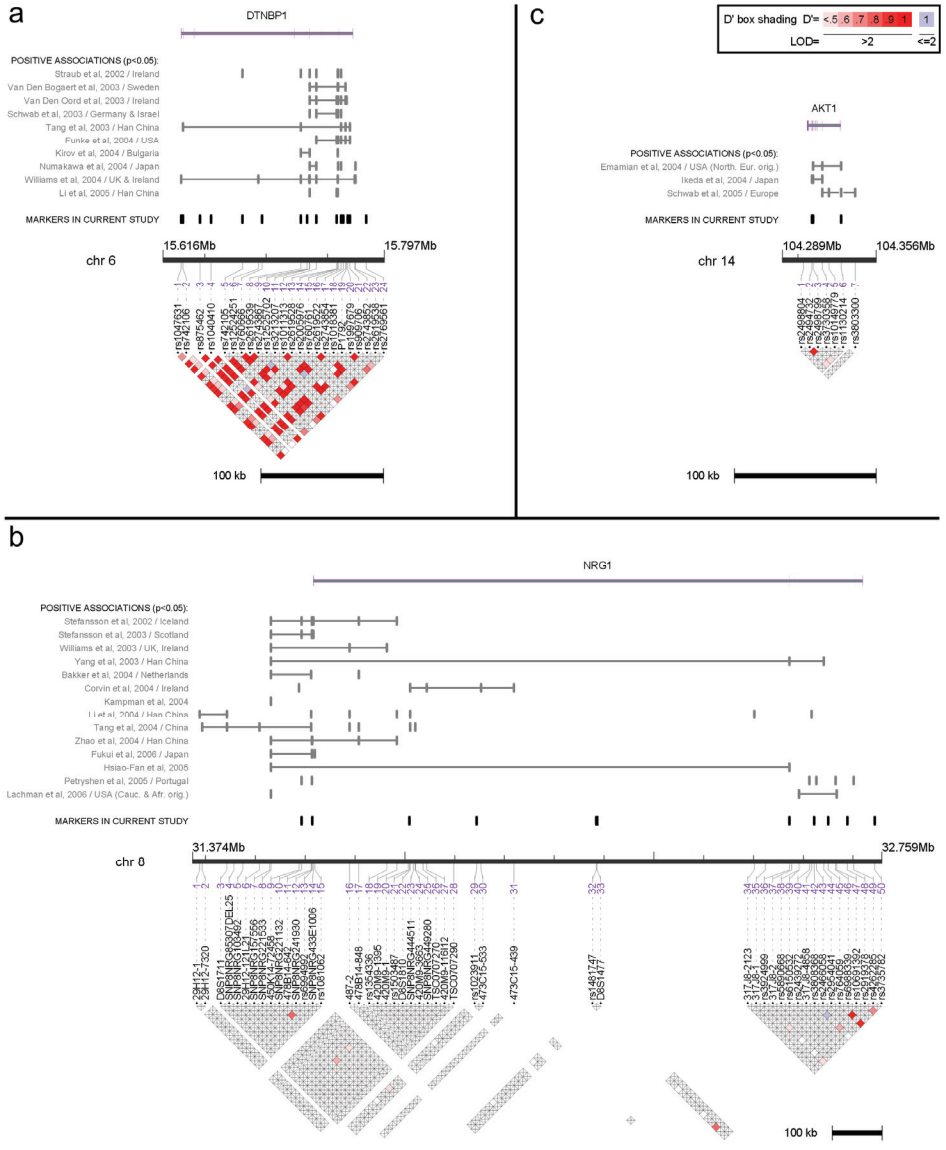
5.3 Gene and marker selection

5.3.1 Study I

In 2002, two reports revealed convincing evidence of association between schizophrenia, and both *DTNBP1* (Straub et al. 2002) and *NRG1* (Stefansson et al. 2002). Importantly, both of these genes are located in previously described schizophrenia loci (Straub et al. 1995, Pulver et al. 1995). Shortly thereafter, several positive replications emerged for both *DTNBP1* (Van Den Bogaert et al. 2003, van den Oord et al. 2003, Schwab et al. 2003, Tang et al. 2003, Funke et al. 2004, Kirov et al. 2004, Numakawa et al. 2004, Williams et al. 2004, Li et al. 2005) and *NRG1* (Stefansson et al. 2003, Williams et al. 2003, Yang et al. 2003, Bakker et al. 2004, Corvin et al. 2004, Kampman et al. 2004, Li et al. 2004, Tang et al. 2004, Zhao et al. 2004, Petryshen et al. 2005, Fukui et al. 2006, Lachman et al. 2006). However, the associated markers and haplotypes varied (Figure 8), and also negative replication attempts were reported. The diverse results encouraged us to study the impact of these genes on schizophrenia in Finland by utilizing our large schizophrenia family sample, especially as a suggestive association between *NRG1* and medication response in schizophrenia had already been reported in a smaller Finnish sample (Kampman et al. 2004). In the initial stage of the study, a novel association was reported between schizophrenia and *AKT1* (Emamian et al. 2004), and shortly thereafter, was replicated in other studies (Ikeda et al. 2004, Schwab et al. 2005), and thus, this small gene was additionally included in the study. The characteristics of these genes are detailed in chapter 2.4.5 and summarized in Table 6.

The intragenic and flanking SNPs were primarily selected among those utilized in the previous studies. To enhance the marker coverage on the genes, additional SNPs were selected from the public database dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), totalling up to 15 SNPs for *DTNBP1*, 10 for *NRG1*, and three for *AKT1*. Of these, eight SNPs for *DTNBP1* were the same as in the original publication (Straub et al. 2002). However, for *NRG1*, only two SNPs were the same as in the original publication (Stefansson et al. 2002). All the three SNPs for *AKT1* were the same as in the original publication (Emamian et al. 2004). Additionally, our existing genotype data were utilized from two flanking STRs for *DTNBP1*, two flanking and one intragenic STRs for *NRG1*, and one flanking STR for *AKT1* (Paunio et al. 2001, Paunio et al. 2004) (Figure 8).

Figure 8. Analyzed SNPs for a) *DTNBP1*, b) *NRG1*, and c) *AKT1* genes (in black), as well as all the SNPs showing association (P<0.05) with schizophrenia in previous studies with the connecting lines indicating the most significantly associated haplotypes (in gray). The marker positions are presented according to the NCBI Build 34 human genome assembly. The relative positions of the gene transcripts are presented at the top. The LD structures in Finns are presented below the marker names. Modified with permission from Figure 1 in study I (Turunen et al. 2007).



5.3.2 Study II

Previously, our genome-wide linkage scan had revealed a novel schizophrenia locus on chromosome 7q22 (Ekelund et al. 2000). Additionally, our genome-wide scan of cognitive schizophrenia endophenotypes had revealed a locus for semantic clustering—a learning strategy—on 7q21 (Paunio et al. 2004). Only a few other studies utilizing samples of European origin have reported even suggestive linkage to chromosome 7q, however, and only one of them to chromosome 7q21-q31 (Moises et al. 1995, Blouin et al. 1998, Faraone et al. 1998). To further evaluate this locus in a larger sample of Finnish schizophrenia families, we utilized altogether 25 STRs on the full length of chromosome 7. The markers had been originally selected from the Cooperative Human Linkage Center (CHLC) 6 set with an average intermarker interval of 7.4 cM. These previously genotyped markers had not been utilized in the full sample before (Paunio et al. 2001, Paunio et al. 2004). Additionally, five intragenic STRs within the *RELN* gene, identified by sequencing by Millennium Pharmaceuticals Inc. (Cambridge, MA, USA) were included. The genetic distances were primarily derived from the Marshfield map (http://research.marshfieldclinic.org/genetics/). For the proprietary intragenic *RELN* STRs, the Human Genome Browser (Kent et al. 2002) was used to estimate the map distances by presuming the equivalence of 1 Mb to 1 cM.

After linkage analysis, altogether four functional and regional candidate genes were selected from chromosome 7q21-32 for further association analysis. The characteristics of the selected genes *SEMA3A*, *GRM3*, and *RELN* are detailed in chapter 2.4.5 and summarized in Table 6. In addition, the *VGF nerve growth factor inducible* (*VGF*) [OMIM 602186] gene, showing a wide pattern of expression in the nervous system (Trani et al. 1995, Salton et al. 2000), especially during the organization of the cerebellum and synaptogenesis (Salton et al. 1991, Lombardo et al. 1995), was included in the study.

The validated intragenic and flanking SNPs, 4 for *GRM3*, 10 for *RELN*, 7 for *SEMA3A*, and 2 for *VGF*, were selected by using the public application SNPper (Riva et al. 2004). The SNPs had to be bi-allelic, validated in Caucasian populations, present in multiple databases, and found with a minor allele frequency (MAF) ≥10%.

5.3.3 Study III

Based on the detected association between *RELN* and cognitive functions in schizophrenia families (II), the *RELN* marker coverage was expanded. Additionally, the number of neuropsychologically assessed subjects had increased after study II, allowing both extension of the sample size and replication efforts.

The previously utilized *RELN* intragenic STRs RELNSAT2 and RELNSAT6 were selected based on the previous schizophrenia linkage and quantitative trait association results, respectively (II), to further genotyping in individuals not previously investigated with these markers. The novel *RELN* promoter-region STR D7S3120 was selected based on previous autism and schizophrenia studies (Fatemi 2005).

The *RELN* intragenic and flanking tagSNPs were selected by using Tagger (de Bakker et al. 2005) implemented in Haploview 4.1 (Barrett et al. 2005) with pairwise tagging method with r^2 threshold of 0.8 based on the CEPH genotype data of the International

HapMap Project (International HapMap Consortium 2005). The tagSNPs had to be bi-allelic with MAF ≥10%.

5.3.4 Study IV

Our previous genome-wide linkage analyses had revealed schizophrenia loci on chromosomes 2q and 5q (Paunio et al. 2001), as well as a locus for visual working memory on chromosome 2q and a locus for verbal learning and memory on chromosome 4q (Paunio et al. 2004). These cognitive traits are considered as valid schizophrenia endophenotypes (Gur et al. 2007). Of these, the 2q and 5q schizophrenia loci have also been highlighted in international meta-analyses (Lewis et al. 2003, Ng et al. 2009). However, these three loci had not been studied previously in an extensive manner in our sample.

The selected chromosomal regions 2q33.1-2q37.3, 4q13.1-4q26, and 5q31.1-5q33.3 were screened for all known genes with a HUGO Gene Nomenclature Committee (HGNC) approved ID by using the Ensembl Human database (Hubbard et al. 2007). Based on literature searches in scientific collaboration with researchers from Orion Pharma, the relevance of the identified 768 genes was evaluated for molecular etiopathogenesis of schizophrenia. The evaluation was based on previously reported associations with schizophrenia and related disorders, the known functions of the genes, and the hypothesized pathophysiology of schizophrenia (Harrison et al. 2005, Rapoport et al. 2005, Burmeister et al. 2008, Keshavan et al. 2008). In all, 104 genes were selected for analysis.

The altogether 1511 intragenic and flanking tagSNPs for the candidate genes were selected by using Tagger (de Bakker et al. 2005) with the pairwise tagging method with r^2 threshold of 0.8 based on the CEPH genotype data of the International HapMap Project (International HapMap Consortium 2005). The tagSNPs had to be bi-allelic with MAF ≥10%. For the six largest genes (*ERBB4*, *HDAC4*, *PPP2R2B*, *GRIA1*, *CAMK2D*, and *GRID2*), an additional two-marker tagging was used with the same threshold values.

5.4 Genotyping

5.4.1 Single nucleotide polymorphisms

In studies I and II, the SNPs were genotyped in 3 to 4-plex reactions in 384-well plates by using Sequenom homogenous Mass Extend (hME) MassARRAY platform according to manufacturer's instructions (Sequenom Inc., San Diego, CA, USA). The flanking DNA sequences of the SNPs were derived from SNPper (Riva et al. 2004), and PCR and extension primers were designed by using Sequenom SpectroDESIGNER 2.0. The PCR reactions were performed in a total reaction volume of 5 µl using 7.5 ng of genomic DNA. As quality controls, eight water controls and eight duplicated DNA samples were included in each plate. The alleles were automatically called by Sequenom MassARRAY Typer and verified manually by two independent reviewers.

For study III, the SNPs were genotyped in 24 to 31-plex reactions in 384-well plates by using Sequenom MassARRAY iPLEX Gold platform according to manufacturer's instructions (Sequenom Inc., San Diego, CA, USA). The flanking DNA sequences of the

SNPs were derived from SNPper (Riva et al. 2004), and PCR and extension primers were designed with Sequenom MassARRAY Assay Design 3.1. The PCR reactions were performed in a total reaction volume of 5 µl using 12.5 ng of genomic DNA. As quality controls, eight water controls and eight duplicated DNA samples were included in each plate. The alleles were automatically called by Sequenom MassARRAY Typer 4.0 and verified manually.

In study IV, The SNPs were genotyped in 96-well plates by using the Illumina GoldenGate platform according to manufacturer's instructions (Illumina Inc., San Diego, CA, USA). The PCR reactions were performed using 50 ng of genomic DNA. As quality controls, one plate-specific and one inter-plate duplicated DNA sample was included in each plate. The alleles were automatically called by Illumina BeadArray Reader and verified manually.

5.4.2 Short tandem repeats

In studies I and II, the STR genotype data were received from previously performed genotyping (Paunio et al. 2001, Paunio et al. 2004).

In study III, the STR genotyping was performed in single-plex reactions in 96-well plates by using ABI 3730xl DNA Analyzer platform (Applied Biosystems, Foster City, CA, USA). The flanking DNA sequences of RELNSAT2 and RELNSAT6 were derived from the UCSC Genome Browser (Kent et al. 2002) and PCR primers were designed with Primer3 software (Rozen et al. 2000). The D7S3120 STR PCR primers were derived from a previous study (Persico et al. 2001). The PCR reactions were performed in a total reaction volume of 15 µl including 10 ng of genomic DNA. As quality controls, two water controls and two duplicated DNA samples were included in each plate. The alleles were automatically called by ABI GeneMapper 4.0 and verified manually.

5.4.3 Quality controls

For all the markers, inclusion criteria included the genotyping success rate ≥95% (II-IV), MAF ≥1% (I-IV), HWE P-value ≥0.01 calculated from non-related individuals by using standard χ^2 test (I), PEDSTATS 0.6.10 (Wigginton et al. 2005) (II, III), or Haploview 4.0 (Barrett et al. 2005) (IV), and the number of Mendelian errors <5 (I-III) or <3 (IV) according to PedCheck 1.1 (O'Connell et al. 1998) (I-III) or PLINK 1.05 (Purcell et al. 2007) (IV). In study I, no marker-specific threshold for success rate was used, however, the overall genotyping success rate was >95%. Additionally, in study II the reliability of the genotyping results was verified with the multipoint error detection option of MERLIN 1.1.2 (Abecasis et al. 2002) identifying possible problematic genotypes using all the genotype information simultaneously.

For the subjects, inclusion criteria included the individual genotyping success rate ≥90%, and the number of Mendelian errors ≤3 per individual and per nuclear family. In case of occasional Mendelian errors, all the genotypes were removed for the corresponding marker and nuclear family (I-IV).

5.5 Statistical analysis

5.5.1 Linkage disequilibrium and haplotype block estimation

The LD between SNPs was estimated among trio founders (II), or among non-related individuals (one per family) (I, III, IV) by using Haploview (Barrett et al. 2005). Additionally, LD between SNPs and STRs was estimated by using ldmax (Excoffier et al. 1995) implemented in GOLD (Abecasis et al. 2000). Haplotype blocks were defined according to the confidence interval algorithm (Gabriel et al. 2002), in which all the SNPs in the haplotype blocks are in strong LD with each other (I), or 'solid spine of LD' algorithm, in which the first and last SNP in every haplotype block are in strong LD with all the intermediate SNPs, which may or may not be in strong LD with each other (II, III). Either the tagSNPs (I-II) or all the markers (III), including the STRs, within the haplotype blocks were used to construct the haplotypes.

5.5.2 Linkage analysis

Singlepoint linkage analyses (II) were performed with Pseudomarker (Goring et al. 2000) which performs separate and joint linkage and LD analyses testing each marker locus against a phenotype-based "pseudomarker" locus. This likelihood-based analysis method, numerically equivalent to model-free analysis, uses efficiently mixed data sets of singletons and various pedigrees. In the linkage analysis, default dominant and recessive models with no phenocopies and low gene frequency were used.

Nonparametric multipoint linkage analyses (II) were performed with SimWalk2 (Sobel et al. 1996, Sobel et al. 2001, Sobel et al. 2002) after preparation of files with Mega2 (Mukhopadhyay et al. 2005), and results were confirmed with MERLIN (Abecasis et al. 2002) by using the helper program AUTOGSCAN (Hiekkalinna et al. 2005).

5.5.3 Qualitative association analysis

In studies I, III, and IV, qualitative allelic association analyses were performed by using FBAT (Laird et al. 2000, Horvath et al. 2001) on affected-only basis. The analysis was performed by using additive model with empirical variance, as recommended in the presence of linkage (Lake et al. 2000), since all the studied genes were located within the previously described schizophrenia loci (Table 5, Table 6). In study II, the allelic qualitative association analyses were performed by using Pseudomarker (Goring et al. 2000) similarly to the linkage analysis. The 'LD given linkage' option was used due to the known presence of linkage in the region.

In studies I and III, qualitative haplotype association analyses were performed with the haplotype analysis option of FBAT (Horvath et al. 2004) in a similar manner as the allelic association analysis. In study II, the haplotype association analysis was performed with TRANSMIT (Clayton 1999) by using bootstrapping method with 100 000 replicates. Alleles and haplotypes with frequencies <3% were pooled together.

In sex-specific analyses, the phenotypes of the other gender were set as unknown.

5.5.4 Quantitative association analysis

Quantitative association analyses were performed with QTDT (Abecasis et al. 2000) with the proportion of alleles shared identical by descent (IBD) calculated with multipoint computation of MERLIN (Abecasis et al. 2002) to extract maximal inheritance information from the families. The total association model, which allows more powerful analysis of samples including partly incomplete families, was used with 'polygenic', 'non-shared environment', 'common environment', and 'nuclear family environment' as variance components, as they supposedly best describe the similarities in the analyzed traits between the family members. The population stratification, a potential source of false positive signals, was determined to be statistically insignificant in our sample with the 'population stratification' model of QTDT (Abecasis et al. 2000) by using the same variance components and covariates as in the association analysis (II-IV).

In the analysis of neuropsychological traits derived from WMS-R (Wechsler 1987), WAIS-R (Wechsler 1981), CVLT (Delis et al. 1987), TMT (Reitan et al. 1993), and Stroop Task (Golden 1978), the used covariates were sex, testing age, and affection status according to any psychotic disorder, since all these factors are related to variations in the overall cognitive performance (Heaton et al. 2001) (II, III). In the analysis of clinical variables derived from SANS (Andreasen 1983), the used covariates were sex, and affection status according to any psychotic disorder, and from SAPS (Andreasen 1984) and OPCRIT (McGuffin et al. 1991), the used covariate was sex (III). Additionally, in study IV, the isolate status (IS/AF) was included as a covariate.

To explore the effect of the correlation between the cognitive functions [tests derived from WMS-R (Wechsler 1987), WAIS-R (Wechsler 1981), CVLT (Delis et al. 1987), TMT (Reitan et al. 1993), and Stroop Task (Golden 1978)], and both the severity of positive symptoms and the severity of negative symptoms of schizophrenia, the sum of all SAPS (Andreasen 1984) or SANS (Andreasen 1983) items were included as covariates in cognitive trait analyses (III). Other covariates were sex, testing age, and additionally, affection status according to any psychotic disorder when severity of negative symptoms was included as a covariate. The analysis model and variance components were the same as in the main analysis.

In study III, the quantitative haplotype association analyses were performed with QTDT (Abecasis et al. 2000) by using the same options as in the allelic association analysis. The haplotypes were constructed with MERLIN (Abecasis et al. 2002) according to the most likely pattern of gene flow, and the haplotypes were coded as numeric alleles by using a custom-made computer script.

5.5.5 Statistical significance estimation

P-value thresholds corresponding to type I error rate of 0.05 were estimated by using conservative Bonferroni correction according to the number of independent markers, and additionally, the number of independent traits (III, IV). Since both the analyzed markers and traits were not fully independent, their effective numbers were estimated with SNPSpD (Nyholt 2004) and matSpD (Nyholt 2004), respectively. The smaller of the pair of estimates, either Meff or MeffLi (Li et al. 2005), or Veff or VeffLi (Li et al. 2005), respectively, were used as recommended by the author (http://gump.qimr.edu.au/general/daleN/SNPSpD/). As for the effective number of independent markers, the SNPSpD (Nyholt 2004) estimate for SNPs (IV), or the sum of the SNPSpD (Nyholt 2004) estimate for SNPs and the number of the STRs (III) was used. As for the effective number of independent traits, the sum of the matSpD (Nyholt 2004) estimates for qualitative and quantitative traits was used (III). The Pearson correlation coefficient matrixes of qualitative and quantitative traits were calculated by using SPSS 16.0.1 (SPSS Inc., Chicago, IL, USA).

In study III, the P-value threshold of 0.0009 for significant and 0.00007 for highly significant trait associations were estimated according to the number of independent markers (n=56.74), and the product (n=717.76) of the number of independent markers (n=56.74) and the number of independent traits (n=12.65), respectively. Additionally, a P-value threshold of 0.01 was used for suggestive association.

In study IV, the P-value threshold of 0.0005 for suggestive and 0.00006 for significant trait associations were estimated according to the number of analyzed genes (n=104) and the effective number of independent markers (n=791.18), respectively.

5.5.6 Effect estimation

In studies II and III, the significance of trait differences between the individuals positive (carrying one or two copies of the allele or haplotype) and negative (not carrying the allele or haplotype) for the STR alleles or haplotypes showing association with the trait were estimated with the Generalized Estimation Equation Model (GEE) (Zeger et al. 1986) of the package 'gee' in R (R Development Core Team 2006). Sex, testing age [with cognitive traits (II, III)] and affection status according to any psychotic disorder [with cognitive traits (II, III), and SANS (III)] were used as covariates. The nuclear family status was used as cluster division to take into account the within-family correlation.

In study IV, the effects of the SNPs showing association with the traits were analyzed by using linear modelling in R (R Development Core Team 2006) with sex, testing age, affection status according to any psychotic disorder, isolate status (AF/IS), and SNP genotype coded as 0, 1, or 2 copies of the minor allele as predictors. For the SANS (Andreasen 1983) sum score, the predictors were sex, affection status according to any psychotic disorder, isolate status (AF/IS), and SNP genotype coded as 0, 1, or 2 copies of the minor allele. For the SAPS (Andreasen 1984) sum score and the age of onset from OPCRIT (McGuffin et al. 1991), the predictors were sex, isolate status (AF/IS), and SNP genotype coded as 0, 1, or 2 copies of the minor allele. In the traits derived from the clinical diagnostic categories and OPCRIT (McGuffin et al. 1991), the effects were estimated by logistic regression in R (R Development Core Team 2006) with predictors

sex, isolate status (AF/IS), and SNP genotype coded as 0, 1, or 2 copies of the minor allele.

For qualitative traits, the effect size was estimated as odds ratio (OR), which describes how likely an incident (here, being affected) is in individuals with the investigated allele compared to individuals without the allele. For quantitative traits, the effect size was estimated as beta value, which describes the quantity of the average trait value change attributed to the investigated allele.

The correlations between the severity of clinical symptoms and amount of risk alleles were estimated by fitting the values into a linear model and calculating the Pearson correlation coefficient in R (R Development Core Team 2006). The markers not in strong LD with each other ($r^2<0.10$) were selected for the analysis by using Tagger (de Bakker et al. 2005) implemented in Haploview (Barrett et al. 2005).

6 Results and discussion

6.1 Analysis of candidate genes *DTNBP1*, *NRG1*, and *AKT1* (Study I)

6.1.1 Association analysis of clinical diagnostic categories

In allelic and haplotype analyses, no associations with P-value <0.01 were detected between any of the studied markers or haplotypes and clinical diagnostic categories. One STR flanking *DTNBP1* (D6S285), and altogether six SNPs and one haplotype within and flanking *NRG1* showed marginal signals (P<0.05), although these cannot be considered statistically significant associations.

For *DTNBP1*, the studied 15 SNPs and 2 flanking STRs cover relatively well the common variants of the gene, and altogether eight of the SNPs were the same as utilized in the original publication revealing association with schizophrenia (Straub et al. 2002). In previous studies, the detected associations have concentrated in the 5' part of the gene. Interestingly, the flanking D6S285 STR (P=0.01 with LC3 in combined sample) is located on this side of the gene. However, despite this gene region having the highest marker coverage in our study as well (Figure 8a), any other even suggestive signals remained absent. The population differences are unlikely to cause the inconsistencies between the studies, however, since the overall haplotype structures of the study populations correspond to each other at a high degree (Mutsuddi et al. 2006).

For *NRG1*, the studied 10 SNPs, and one intragenic and two flanking STRs are supposedly insufficient to cover other than the most common variants of the gene due to its large size, although the high LD in Finnish samples compensates the low number of the markers for some degree (Service et al. 2006). Although the studied SNPs included two from the original publication revealing association with schizophrenia (Stefansson et al. 2002), our marker coverage concentrated in the 3' part of the gene with lower number of reported associations (Figure 8b). However, any association with the SNPs belonging to the original "Icelandic" haplotype in the 5' part of the gene, its most studied region, remained absent. On the other hand, the detected suggestive association signals from the 3' region of the gene could be interpreted, in combination with the previous evidence, as supportive for the involvement of this region in the etiology of schizophrenia (Yang et al. 2003, Li et al. 2004, Petryshen et al. 2005, Lachman et al. 2006). Furthermore, these multiple marginal signals emerged among the AF subsample from the geographical region of Finland, in which the genetic architecture is more closely related to other Western European populations than that in the isolate (IS) located in the northeastern Finland (Salmela et al. 2008).

For *AKT1*, the studied three SNPs and one flanking STR are supposedly sufficient to cover the common variants of this gene due to its small size (Figure 8c). All these SNPs overlap with those investigated in the original publication revealing association with schizophrenia (Emamian et al. 2004).

6.1.2 Conclusions

Overall, this study showed no support for any major role for *DTNBP1*, *NRG1*, or *AKT1* in the genetic liability of schizophrenia in Finland. However, the number of analyzed genetic markers, 15 SNPs for *DTNBP1*, 10 for *NRG1*, and 3 for *AKT1*, was relatively low. This leaves a possibility for other regions of these genes playing a role behind the liability. On the other hand, the SNPs in our study were selected primarily based on the previous studies, and therefore, the selection of the SNPs cannot solely explain the negative results in our series.

Despite the lacking significant associations in this study, multiple line evidence suggest a role for the studied genes in schizophrenia in other populations. For *DTNBP1*, this evidence includes the location of *DTNBP1* in schizophrenia-linked region (Straub et al. 1995), association with schizophrenia (Straub et al. 2002) and cognitive features (Donohoe et al. 2007), and the reduced level of *DTNBP1* expression in schizophrenia (Weickert et al. 2004, Weickert et al. 2008). Despite the negative association results in this study, *DTNBP1* remains as one of the notable candidate genes for schizophrenia. For *NRG1*, the established roles in the development and function of the brain (Harrison et al. 2006), the location in schizophrenia-linked region (Pulver et al. 1995), and association with schizophrenia (Stefansson et al. 2002) propose its actions behind the schizophrenia pathogenesis. Similarly, the AKT pathway seems to be associated with both the liability of schizophrenia (Emamian et al. 2004), and with that of bipolar disorder (Toyota et al. 2003). In murine model, the Akt pathway is associated with spatial memory functions (Mizuno et al. 2003), and interestingly, human *AKT1* is associated with verbal learning and memory, as well as with cortical gray matter density (Pietilainen et al. 2009).

To conclude, our study is insufficient to fully exclude the role of these genes in the pathogenesis of schizophrenia or closely related traits, especially as our phenotypic information were limited to clinical diagnosis only. Therefore, only more detailed analysis with novel markers and/or phenotypic features may elucidate the role of these genes in Finnish schizophrenia families.

6.2 Analyses of 7q22 locus and regional candidate gene *RELN* (Studies II & III)

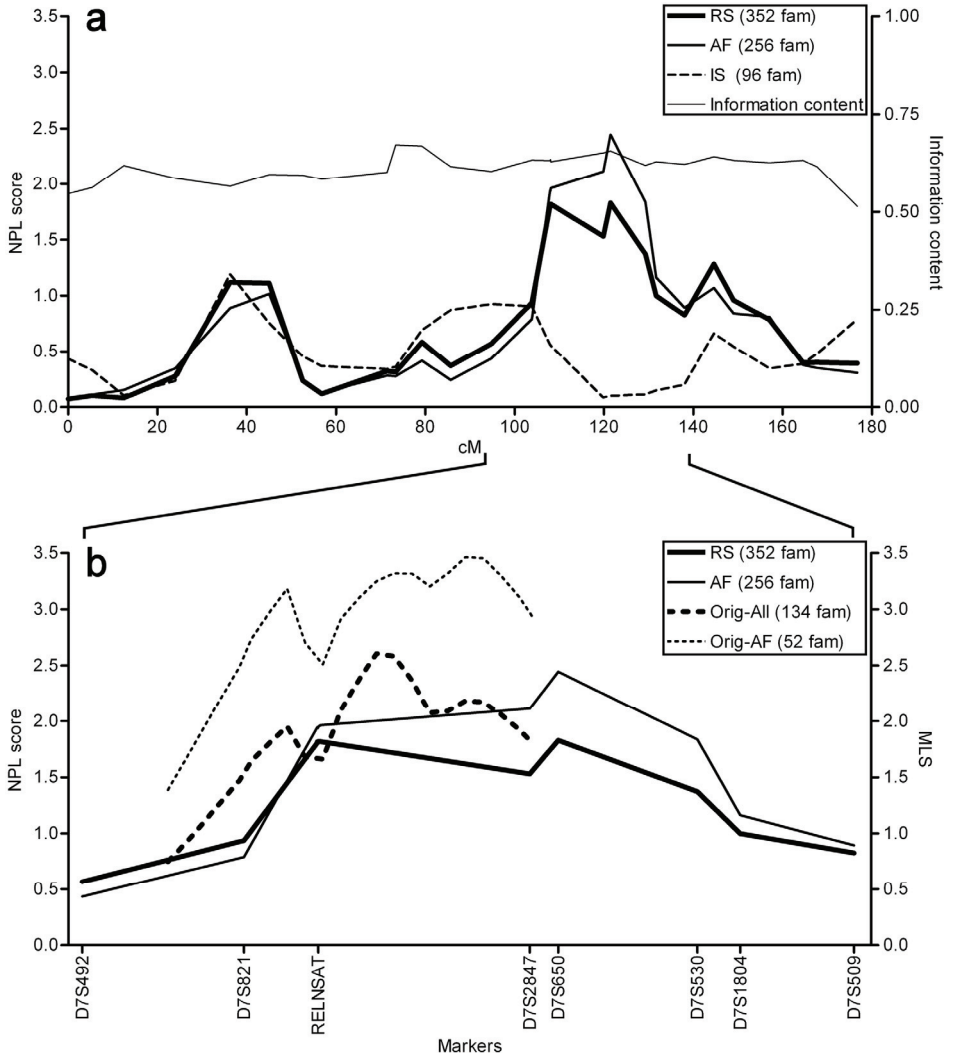
6.2.1 Linkage analysis of clinical diagnostic categories

The singlepoint linkage analysis was performed among the replication sample (RS) of altogether 352 families independent from our previous study (Ekelund et al. 2000). The highest LOD scores were 0.65 for STR D7S2204 when using core schizophrenia (LC1) as a diagnostic criterion, and 0.82 for *RELN* intragenic STR RELNSAT2 when using schizophrenia spectrum (LC3).

In multipoint analysis, the highest SimWalk2 multipoint NPL scores of 1.38 (LC1) and 1.83 (LC3) were detected between the STRs D7S821 (7q21.3) and D7S1804 (7q32.3). Among the subsamples of 256 AF families and 96 IS families, the AF subsample revealed the highest SimWalk2 multipoint NPL score of 2.44 between the STRs D7S821 (7q21.3) and D7S1804 (7q32.3) with the LC3 criterion. The IS subsample revealed no significant linkage to chromosome 7 (Figure 9).

The confirmation analyses with MERLIN revealed consistently similar patterns of linkage in all classes (LC1, LC3) (data not shown). Thus, we replicated the schizophrenia linkage on chromosome 7q21-q32 in an independent series of 352 Finnish nuclear families, and in agreement with the original study (Ekelund et al. 2000), this signal was predominantly contributed by the families outside the internal isolate.

Figure 9. a) SimWalk2 multipoint linkage analysis results (LC3) in the replication sample (RS), the subsample outside the isolate (AF), and the isolate subsample (IS) on non-parametric linkage (NPL) score. b) Magnification of the linked region with the marker positions indicated. RELNSAT refers to all the five *RELN* intragenic STRs. Additionally, our original multipoint linkage results (LC3) (Ekelund et al. 2000) are shown on the maximum likelihood score (MLS) scale for the whole original sample (Orig-All), and for its subsample originating outside the isolate (Orig-AF). Modified with permission from Figure 1 in study II (Wedenoja et al. 2008).



6.2.2 Association analysis of clinical diagnostic categories

None of the markers or haplotypes in the selected regional and functional candidate genes *SEMA3A*, *GRM3*, *VGF*, and *RELN* showed significant association with any of the diagnostic categories among the families originating from the same geographical region as those revealing the linkage to chromosome 7q21-q32. Similarly, the sex-specific analyses failed to reveal any association (II). Further evaluation of *RELN* with extended marker coverage in the partly overlapping sample, including also nuclear families originating from the internal isolate (Hovatta et al. 1997), showed no significant associations with any of the markers or haplotypes (III).

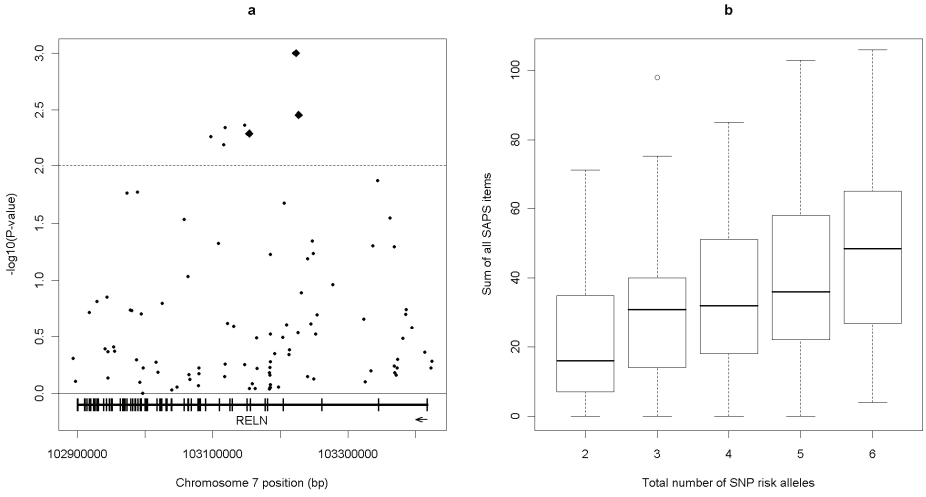
The *RELN* promoter-region STR D7S3120 alleles and their frequencies resembled those reported previously (Persico et al. 2001). In our Finnish sample, however, none of the alleles exceeded the length of 15 repeats (III). This is noteworthy, since the longer alleles are associated with autism (Persico et al. 2001) and treatment-resistant schizophrenia (Goldberger et al. 2005), possibly via decreased *RELN* expression (Persico et al. 2006).

6.2.3 Association analysis of clinical disorder features

Suggestive associations ($P<0.01$) emerged between the severity of positive symptoms and SNPs rs2299356, rs12705141, rs727708, rs540058, rs563264, rs16872603, and rs11761011 (Figure 10a) (III). All the identified SNP risk alleles increased the symptom severity in an additive fashion (data not shown). After selecting the SNPs not in strong LD with each other ($r^2<0.10$; rs563264, rs16872603, and rs11761011) by using Tagger (de Bakker et al. 2005) implemented in Haploview 4.1 (Barrett et al. 2005), the total number of their risk alleles and the severity of positive symptoms showed a significant positive correlation ($P=0.0000001$, $r=0.24$) (Figure 10b).

Among the affected individuals, the longest allele of RELNSAT6 (AATA repeat in *RELN* intron 27) showed significant association with both more severe positive symptoms ($P=0.0005$; Beta 13.28, 95%CI 3.74-22.82) and negative symptoms ($P=0.00006$; Beta 16.50, 95%CI 7.17-25.83) of schizophrenia (III). Additionally, the allele 13 of D7S3120 showed suggestive association ($P=0.002$) with the age of schizophrenia onset, the allele positive individuals (n=23) having earlier age of onset (mean 20.3, median 18 years) than the allele negative individuals (n=432) (mean 24.6, median 22 years).

Figure 10. a) SNP association results with the severity of positive symptoms of schizophrenia as a sum of all SAPS (Andreasen 1984) items. The horizontal dashed line corresponds to P=0.01. The SNPs rs2299356, rs12705141, rs727708, rs540058, rs563264, rs16872603, and rs11761011 showed suggestive association with the severity (P<0.01). Of these, the SNPs rs563264, rs16872603, and rs11761011 (presented as diamonds) were selected to further analysis by using the Tagger algorithm (de Bakker et al. 2005) implemented in Haploview 4.1 (Barrett et al. 2005).
b) The severity of the positive symptoms compared with the total number of risk alleles of those three SNPs. Only the individuals with genotypes available for all the three SNPs were included in the analysis. The number of individuals in each category (category in parentheses): 25 (2); 106 (3); 186 (4); 125 (5); 30 (6). Due to the low number of individuals, the categories one and two were combined. The rectangle ends in the boxplots represent the lower (Q1) and upper (Q3) quartiles, the horizontal lines in the rectangles the median values, the whiskers the smallest and largest non-outlier values [lower limit: Q1-1.5*(Q3-Q1)); upper limit: Q3+1.5*(Q3-Q1)], and the circles the outlier values. Modified with permission from Figure 1 in study III (Wedenoja et al. 2009).



6.2.4 Association analysis of neuropsychological test measurements

In the analysis of quantitative traits derived from neuropsychological test measurements (II), RELNSAT6 showed suggestive global association with verbal working memory (P=0.003). In further allele-specific analysis, the longest detected RELNSAT6 allele—hence, the risk allele—showed significant association with several traits. The allele positive individuals (those carrying one or two copies of the allele, n=23) showed generalized cognitive impairment compared with the allele negative individuals (those not carrying the allele, n=527). In replication analysis among altogether 342 individuals with genotype and trait information available (III), this original STR association was replicated with the allele positive individuals (n=20) showing similarly impaired cognitive performance as in the original study (Table 10). Interestingly, the effect of the

risk allele was stronger among the individuals affected with psychosis (ANYPSY criteria) (Table 11).

To further diminish the effects of within-family correlation and age, the performance differences were further explored by selecting only the youngest risk allele positive (n=12) or negative (n=169) individual from each family (II). Among these unrelated individuals, the performance differences remained significant for verbal (P=0.005) and visual (P=0.003) working memory, and verbal memory (P=0.02) traits according to the Mann-Whitney U-test, and the overall trend in test performance remained for all the tested traits (data not shown).

Since the neuropsychological traits and both the SAPS (Andreasen 1984) and SANS (Andreasen 1983) symptom ratings showed correlation (Table 12), the effect of this correlation on the results was further explored by including the SAPS and SANS sum scores as covariates. The association between the risk allele and verbal working memory remained suggestive (P=0.003) with SAPS included as covariate, and significant (P=0.0003) with SANS included as covariate, despite the lower number of informative individuals in these analyses (n=461 and n=627, respectively).

In the AF subsample, the same RELNSAT6 risk allele showed similar patterns of association as observed in the whole sample (II, III). In addition, one allele of RELNSAT7, showing some LD with RELNSAT6 (D'=0.78), revealed significant association with verbal working memory (P=0.003) and visual attention (P=0.0009) (II). In the AF subsample, the frequency of the trait-associated allele was 41.2%. When the AF subsample was divided into homozygous RELNSAT7 allele positives (n=49), heterozygous allele positives (n=147), and allele negatives (n=101), significant difference arose only between the homozygous allele positive and negative individuals for the verbal working memory (P=0.01). Additionally, one haplotype not involving RELNSAT6 showed highly significant association with poorer performance in verbal memory traits (P=0.00005 to 0.00006) (III).

In the IS subsample, the RELNSAT6 risk allele was almost entirely absent; it was detected in only one nuclear family fulfilling the criterion of at least one maternal and one paternal grandparent born in the geographical isolate region (III). In agreement with this and the lack of schizophrenia linkage in the IS subsample (Figure 9), any significant *RELN* associations remained absent in this subsample.

In the control sample, two SNP haplotypes showed association with verbal working memory (P=0.0005), and verbal memory (P=0.0009) (III).

In the total RS sample of altogether 1626 individuals from 352 nuclear families, 54.1% of the RELNSAT6 risk allele positives, compared to 39.6% of the negatives, were affected with schizophrenia spectrum disorders (LC3). This difference was statistically significant according to the χ^2 test (P=0.01).

Table 10. The RELNSAT6 risk allele effects on overlapping neuropsychological test traits (raw test scores) in the original sample (II), and replication subsample (III). Significance is estimated with GEE (Zeger et al. 1986) analysis by using sex, testing age, and affection status according to any psychotic disorder (LC3 in II, ANYPSY in III) as covariates. The nuclear family status was used as cluster division to take into account the within-family correlation. All P-values <0.05 are shown.

Trait	**Original ind (n=550)**		**Replication ind (n=342)**	
Attention and working memory	**Beta (95% CI)**	**P-value**	**Beta (95% CI)**	**P-value**
Verbal attention[a]	-0.69 (± 0.69)	0.03	-0.23 (± 0.69)	ns
Verbal working memory[a]	-1.66 (± 0.53)	$4*10^{-10}$	-0.98 (± 0.93)	0.02
Visual attention[a]	-0.86 (± 0.69)	0.007	-0.88 (± 0.69)	0.007
Visual working memory[a]	-1.59 (± 0.93)	0.0004	-1.36 (± 1.21)	0.01
Verbal learning and memory				
Verbal learning[a]	-6.71 (± 5.72)	0.01	-4.18 (± 5.21)	ns
Recalling words after short delay[a]	-2.31 (± 1.24)	0.0001	-1.05 (± 1.33)	ns
Recalling words after long delay[a]	-1.25 (± 1.15)	0.02	-0.74 (± 1.37)	ns
Ability functions				
Verbal ability[a]	-5.62 (± 5.38)	0.02	-1.18 (± 6.16)	ns
Executive functions				
Stroop interference score[b,c]	13.67 (± 11.00)	0.001	-3.46 (± 13.94)	ns

[a]Lower test score represents poorer performance
[b]Higher test score represents poorer performance
[c]Significance was estimated by using the transformed raw test score
Abbreviations: ind, individuals; CI, confidence interval; ns, not significant

Table 11. The RELNSAT6 risk allele effects on neuropsychological test traits (raw test scores) in the whole schizophrenia sample, and in a subsample of individuals affected with psychosis. Significance is estimated with GEE (Zeger et al. 1986) analysis by using sex, testing age, and affection status according to any psychotic disorder (ANYPSY) as covariates. The nuclear family status was used as cluster division to take into account the within-family correlation. All P-values <0.05 are shown.

Trait	**All ind (n=892)**		**Affected ind (n=385)**	
Attention and working memory	**Beta (95% CI)**	**P-value**	**Beta (95% CI)**	**P-value**
Verbal attention[a]	-0.45 (± 0.50)	0.04	-1.10 (± 0.73)	0.001
Verbal working memory[a]	-1.34 (± 0.54)	$6*10^{-7}$	-1.72 (± 0.76)	$4*10^{-6}$
Visual attention[a]	-0.84 (± 0.48)	0.0003	-1.10 (± 0.74)	0.002
Visual working memory[a]	-1.44 (± 0.82)	0.0003	-1.93 (± 1.35)	0.002
Mental tracking[b,c]	4.21 (± 9.89)	ns	5.85 (± 13.00)	ns
Verbal learning and memory				
Verbal learning[a]	-5.31 (± 4.29)	0.008	-7.88 (± 5.01)	0.001
Recalling words after short delay[a]	-1.58 (± 1.02)	0.001	-2.18 (± 1.42)	0.001
Recalling words after long delay[a]	-0.97 (± 0.97)	0.03	-1.38 (± 1.37)	0.02
Semantic clustering ratio[a]	0.04 (± 0.24)	ns	-0.06 (± 0.39)	ns
Ability functions				
Verbal ability[a]	-3.74 (± 4.32)	0.04	-7.12 (± 6.22)	0.01
Processing speed[a]	-1.22 (± 3.67)	ns	-6.40 (± 4.63)	0.003
Executive functions				
Executive functioning[b,c]	17.90 (± 28.62)	ns	57.86 (± 49.84)	0.0003
Stroop interference score[b,c]	5.86 (± 10.10)	ns	9.50 (± 17.31)	ns

[a]Lower test score represents poorer performance
[b]Higher test score represents poorer performance
[c]Significance was estimated by using the transformed raw test score
Abbreviations: ind, individuals; CI, confidence interval; ns, not significant

Table 12. Spearman's rho values between neuropsychological tests, and the severity of both the positive and negative symptoms of schizophrenia (sum of all SAPS (Andreasen 1984) and SANS (Andreasen 1983) items, respectively).

Trait	Spearman's rho	
Attention and working memory	**SAPS**	**SANS**
Verbal attention	-0.102	-0.257
Verbal working memory	-0.243	-0.415
Visual attention	-0.127	-0.382
Visual working memory	-0.129	-0.365
Mental tracking	0.154	0.458
Verbal learning and memory		
Verbal learning	-0.356	-0.596
Recalling words after short delay	-0.290	-0.516
Recalling words after long delay	-0.285	-0.544
Semantic clustering ratio	-0.253	-0.285
Ability functions		
Verbal ability	-0.179	-0.453
Processing speed	-0.149	-0.496
Executive functions		
Executive functioning	0.161	0.377
Stroop interference score	0.212	0.345

Abbreviations: SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the Assessment of Negative Symptoms

6.2.5 Conclusions

Multiple *RELN* SNPs showed association with the severity of positive symptoms of schizophrenia, and the total number of the risk alleles revealed a positive correlation with the severity. Overall, the strongest signals emerged from the longest allele of RELNSAT6, this allele being associated with impaired cognitive functioning on multiple domains, and with more severe positive and negative symptoms of schizophrenia. As expected, the symptom ratings and cognitive traits showed correlation, but the association with cognition remained while covarying for the symptom ratings. Whether RELNSAT6 itself is the causative variant or merely reflects the effects of another variant(s) remains unclear. That the longest variant revealed the associations, however, could imply the actions of this STR in conjunction with some epigenetic regulatory effects. Interestingly, this kind of phenomenon is seen in some other disorders, most notably the Fragile X Syndrome, where the expansion of a nucleotide repeat exposes the sequence to excess methylation, eventually silencing the expression of the underlying gene (Graff et al. 2008, Graff et al. 2009).

The risk allele appeared only in four families from the internal isolate (IS) (Hovatta et al. 1997), yet only one of the families fulfilled the criterion of at least one maternal and paternal grandparent born in the geographical isolate region. In agreement with this, no *RELN* associations were detected in the IS subsample. Thus, the geographical distribution of *RELN* associations was uneven, supporting the evidence of different risk variants and/or their different frequencies affecting the schizophrenia liability in the isolate and in the general population (Hovatta et al. 1997) (III). That the *RELN*

associations were mostly contributed by the AF families supports the generalizability of our results, since the genetic architecture outside the internal isolate is more closely related to that of the other Western European populations (Salmela et al. 2008).

Although statistical bias due to the low number of risk allele positive individuals (n=42) is possible, the risk allele positives in the non-overlapping individuals between the studies II and III had similar deficits in cognitive performance (Table 10). Interestingly, the effect of the risk allele on cognition was stronger among the cases (Table 11), whereas any effect among the controls remained negligible. This phenomenon fits the modifier gene hypothesis, and proposes a role for *RELN* in modifying the disorder features (Nadeau 2001, Fanous et al. 2005).

Although our studies involved genetic association analyses without functional experiments, a wide spectrum of data supports the role of *RELN* in both development and function of the brain. *RELN* seems to regulate neuronal migration and affect synaptic plasticity (Rice et al. 2001, Fatemi 2005). As for *RELN* structure, its C-terminus is crucial for the downstream signaling (Nakano et al. 2007) and the reelin repeats five and six form the essential binding sites for the Reelin receptors (Yasui et al. 2007). Although the RELNSAT6, showing the strongest signals in our study, is located apart from these supposedly most important functional regions, this location cannot exclude its possible effects in the complex process of gene regulation. That the reduced *RELN* expression appears in several major neurodevelopmental disorders suggest its central role in these conditions (Fatemi 2005). Especially interesting is that the promoter-region hypermethylation of *RELN*, which reduces its expression (Chen et al. 2002), arises in the brains of schizophrenia patients (Grayson et al. 2005). On the other hand, one of the factors increasing the *RELN* methylation may be aging (Tamura et al. 2007). That the trinucleotide repeat lengthening in the *RELN* promoter-region, affecting its expression levels (Persico et al. 2006), appears in treatment-resistant schizophrenia (Goldberger et al. 2005) provides further evidence for *RELN* actions in the background of schizophrenia. Interestingly, a long allele of this *RELN* promoter-region STR showed association in our study with the earlier age of schizophrenia onset.

Despite the fact that the promoter-region of *RELN* may be associated with schizophrenia, other findings of *RELN* associations with schizophrenia are rare (Kahler et al. 2008, Shifman et al. 2008). Thus, regarding the association analysis with clinical diagnoses, our results are in line with the previous evidence. Our study shows, however, that utilization of intermediate phenotypes reveals specific disorder domains associated with *RELN*. While our results add data to the *RELN* literature and support its role especially in cognitive features affected in schizophrenia, the underlying mechanisms between the *RELN* and the disorder liability remain to be clarified. To assist future studies based on our results, we have included all the required information for *RELN* intragenic STR genotyping in the supplementary material of our published article.

6.3 Analysis of 104 regional candidate genes from 2q, 4q, and 5q loci (Study IV)

6.3.1 Chromosome 2q33.1-2q37.3

Our previous studies revealed linkage between chromosome 2q and both schizophrenia (Paunio et al. 2001) and visual working memory (Paunio et al. 2004). The schizophrenia linkage was contributed mostly by the families originating from the internal isolate (IS), whereas working memory linkage was contributed mostly by the families originating outside the isolate (AF).

In this study, 37 regional candidate genes were selected for detailed association analyses of clinical diagnostic categories, clinical disorder features, and cognitive traits. All the detected significant and suggestive associations are summarized in Table 13. The significant and those suggestive associations considered most promising are presented in detail below.

The *CASP8 and FADD-like apoptosis regulator* (*CFLAR*) [OMIM 603599] on chromosome 2q33.1 showed significant association with executive functioning in the control sample (CTRL). Interestingly, *CFLAR* downregulation induces Akt activation via phosphorylation (Shim et al. 2007).

The *V-erb-a erythroblastic leukemia viral oncogene homolog 4* (*ERBB4*) [OMIM 600543] on chromosome 2q34 showed suggestive associations with eight different SNPs and several traits, including widespread delusions (ALL), severity of positive symptoms of schizophrenia (IS), as well as verbal (ALL and IS, different SNPs) and visual attention (IS). Interestingly, a previous study (Nicodemus et al. 2006) has revealed an association with verbal attention among healthy controls with the same Digit Span subtest from WMS-R (Wechsler 1987) which revealed the association here. The characteristics of *ERBB4* are detailed in chapter 2.4.5 and summarized in Table 6.

The *EPH receptor A4* (*EPHA4*) [OMIM 602188] on chromosome 2q36.1 showed suggestive associations with both schizophrenia spectrum (SCHSPECT) and psychotic disorders (ANYPSY) in the whole schizophrenia sample (ALL). Additionally, associations were detected between *EPHA4* and poor premorbid work adjustment (ALL; significant and suggestive), poor premorbid social adjustment (ALL and IS; suggestive; different SNPs), mood symptoms (IS; suggestive), and visual working memory (IS; suggestive). *EPHA4* encodes for an ephrin receptor from the protein-tyrosine kinase family, and is important regulator of the formation of neuronal connections in the brain, especially in the hippocampus (Murai et al. 2003, Ho et al. 2009).

The *Paired box 3* (*PAX3*) [OMIM 606597] on chromosome 2q36.1 showed significant association with long delay verbal recall (AF). *PAX3* is expressed only during the embryogenesis, especially in the developing neuronal tissue (Goulding et al. 1991). *PAX3* mutations cause the Waardenburg syndrome type I and III (Hoth et al. 1993), characterized by several defects in neural crest derived tissues, and especially congenital deafness (Morell et al. 1997).

Table 13. Association results on chromosome 2q33.1-2q37.3. Beta values are for the untransformed trait. Only P-values below the suggestive threshold are shown, and P-values below the significant threshold are shown in bold.

SNP	Gene	Allele[a]	P-value	Trait	OR	Beta	Sample
rs2110728	***CFLAR***	**G**	**0.00006**	**EF**		**11.61**	**CTRL**
		G	0.0001	PS		-4.08	CTRL
rs1983343	*NRP2*	A	0.0003	PWA	1.33		IS
rs1996412	*NRP2*	G	0.0002	S		8.50	IS
rs888085	*NDUFS1*	A	0.0004	MT		4.96	ALL
rs12694040	*NDUFS1*	A	0.0004	MT		4.96	ALL
rs12466841	*KLF7*	A	0.00009	VeAb		-3.37	CTRL
rs13005841	*ERBB4*	A	0.0005	VeAt		-0.49	IS
rs7607942	*ERBB4*	G	0.0004	SAPS		7.53	IS
rs12694261	*ERBB4*	A	0.0001	VeAb		-5.69	CTRL
rs12373751	*ERBB4*	A	0.0004	VeAt		-0.40	ALL
rs1521657	*ERBB4*	A	0.0003	SAPS		8.22	IS
rs1357141	*ERBB4*	G	0.0002	EF		22.63	AF
rs17418814	*ERBB4*	C	0.0004	ViAt		-0.53	IS
rs12104818	*ERBB4*	A	0.0004	WD	1.49		ALL
rs13652	*FN1*	G	0.0004	SDe		-1.40	CTRL
rs645163	*CYP27A1*	G	0.0001	L		-7.30	AF
		G	0.0003	SDe		-1.81	AF
		G	0.0004	LDe		-1.86	AF
rs10498110	*EPHA4*	G	0.0003	SCHSPECT	1.66		ALL
		G	0.0005	ANYPSY	1.51		ALL
		G	0.0002	PWA	1.74		ALL
rs12476016	*EPHA4*	G	0.0005	PSA	1.22		IS
		G	0.0002	MS	1.86		IS
rs9288569	***EPHA4***	**G**	**0.00006**	**PWA**	**1.46**		**ALL**
		G	0.0005	PSA	1.30		ALL
rs2056290	*EPHA4*	G	0.0005	ViWM		-0.53	IS
rs6706608	***PAX3***	**A**	**0.00006**	**LDe**		**-1.37**	**AF**
rs935025	*ACSL3*	G	0.0003	SAPS		10.60	ALL
rs7608941	*SERPINE2*	C	0.0003	VeAb		-5.02	AF
rs7590948	*SERPINE2*	A	0.0002	VeWM		-0.34	ALL
		A	0.0005	VeWM		-0.46	AF
rs4674842	*SERPINE2*	A	0.0004	VeWM		-0.39	ALL
rs13013387	*SERPINE2*	A	0.0002	VeWM		-0.51	ALL
rs3791480	*HDAC4*	G	0.00008	PS		-4.60	AF
rs3732341	*KIF1A*	A	0.0003	VeAb		-2.70	AF
rs11693670	*KIF1A*	C	0.0004	VeWM		-0.33	ALL
rs12624059	*KIF1A*	G	0.0004	VeWM		-0.36	ALL

[a]Allele for which the effect is shown

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; A, adenine; C, cytosine; G, guanine; ALL, whole sample; IS, internal isolate; AF, all Finland (outside the isolate); CTRL, control sample; for gene name abbreviations, refer to the Abbreviations chapter; for trait abbreviations, refer to Table 9

6.3.2 Chromosome 4q13.1-4q26

Our previous study revealed linkage between chromosome 4q and multiple verbal memory traits, contributed mostly by families outside the internal isolate (AF) (Paunio et al. 2004). Additionally, southern families from the internal isolate (IS-S) have revealed linkage between 4q and schizophrenia spectrum disorders (Paunio et al. 2008).

In this study, 36 regional candidate genes were selected for detailed association analyses of clinical diagnostic categories, clinical disorder features, and cognitive traits. All the significant and suggestive associations detected in this study are summarized in Table 14. The significant and those suggestive associations considered most promising are presented in detail below.

The *EPH receptor A5* (*EPHA5*) [OMIM 600004] on chromosome 4q13.1 showed suggestive association with mental tracking (ALL), as well as with short and long delay verbal recall (AF). Like *EPHA4*, also *EPHA5* encodes for an ephrin receptor from the protein-tyrosine kinase family, and regulates synaptogenesis in the hippocampus, and dopaminergic pathways in the midbrain (Martinez et al. 2005, Cooper et al. 2009). In mice, *EPHA5* null mutation causes behavioral changes, such as reduced overall activity (Mamiya et al. 2008). Interestingly, inhibition of EphA receptors leads to impaired performance in hippocampal-dependent memory tasks, whereas Epha5 activation reduces these deficits (Murai et al. 2002).

The *Nucleosome assembly protein 1-like 5* (*NAP1L5*) [OMIM 612203] on chromosome 4q22.1 showed significant (IS) and suggestive association (ALL) with executive functioning with the same SNP. *NAP1L5* is widely expressed in mouse hypothalamus, hippocampus, and cerebral cortex (Davies et al. 2004).

The *Tachykinin receptor 3* (*TACR3*) [OMIM 162332] on chromosome 4q24 showed association with processing speed (CTRL). *TACR3* is expressed widely in the brain (Pinto et al. 2004), and is associated with nicotine and cocaine dependence (Foroud et al. 2008), but at least in one reported study any association with schizophrenia remained absent (Saito et al. 2008).

Table 14. Association results on chromosome 4q13.1-4q26. Beta values are for the untransformed trait. Only P-values below the suggestive threshold are shown, and P-values below the significant threshold are shown in bold.

SNP	Gene	Allele[a]	P-value	Trait	OR	Beta	Sample
rs11737238	*EPHA5*	C	0.0005	SDe		-0.90	AF
rs9999552	*EPHA5*	A	0.0003	SDe		-1.05	AF
		A	0.0001	LDe		-1.06	AF
rs4518304	*EPHA5*	G	0.0005	MT		5.77	ALL
rs7655988	*EPHA5*	A	0.0004	LDe		-1.00	AF
rs7670819	*UGT2A1*	A	0.00008	PWA	1.81		AF
rs1560605	*UGT2A1*	C	0.0005	PWA	1.46		AF
rs11937245	*SLC4A4*	G	0.0003	EF		20.59	IS
rs12507157	*EREG*	G	0.0003	ANYPSY	1.12		IS
rs17262520	*SEPT11*	C	0.0003	S		6.89	ALL
rs878729	*SEPT11*	G	0.0002	S		7.31	ALL
rs6535252	*RASGEF1B*	A	0.00009	ViWM		-0.59	IS
rs2972011	***NAP1L5***	A	0.0004	EF		11.48	ALL
		A	**0.00003**	**EF**		**19.28**	**IS**
rs1394342	*GPRIN3*	G	0.0002	EF		15.75	ALL
rs223391	*ZCD2*	G	0.0002	S		5.40	ALL
rs13134657	***TACR3***	**C**	**0.00002**	**PS**		**-6.95**	**CTRL**
rs11098195	*CAMK2D*	A	0.0004	SANS		4.39	ALL

[a]Allele for which the effect is shown

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; A, adenine; C, cytosine; G, guanine; ALL, whole sample; IS, internal isolate; AF, rest of Finland (outside the isolate); CTRL, control sample; for gene name abbreviations, refer to the Abbreviations chapter; for trait abbreviations, refer to Table 9

6.3.3 Chromosome 5q31.1-5q33.3

Our previous study revealed linkage between chromosome 5q region and both schizophrenia and the broad category of severe major affective disorders, both contributed mostly by the families originating outside the internal isolate (AF) (Paunio et al. 2001). However, no linkage was detected with the cognitive traits (Paunio et al. 2004).

In this study, 31 regional candidate genes were selected for detailed association analyses of clinical diagnostic categories, clinical disorder features, and cognitive traits. All the significant and suggestive associations detected in this study are summarized in Table 15. The significant and those suggestive associations considered most promising are presented in detail below.

The associations with any of the clinical diagnostic categories remained non-existent. However, the *RAD50 homolog* (*RAD50*) [OMIM 604040] on chromosome 5q31.1 showed significant association with the age of schizophrenia onset in the AF subsample. The associating marker was, however, not intragenic but flanking 28 kb. RAD50 is an essential part of a protein complex central in DNA repair (Lee et al. 2005).

Interestingly, multiple significant associations emerged with the cognitive traits. The *Purine-rich element binding protein A* (*PURA*) [OMIM 600473] on chromosome 5q31.3 showed association with short delay verbal memory in the AF subsample. *PURA* encodes for DNA- and RNA-binding protein related to cell cycle (Gallia et al. 2000). This protein is essential in postnatal brain development (Khalili et al. 2003). Additionally, *PURA* shows altered expression in bipolar I disorder (Nakatani et al. 2006).

The *Heparin-binding EGF-like growth factor* (*HBEGF*) [OMIM 126150] on chromosome 5q31.3 showed significant association with short delay verbal memory in the AF subsample. *HBEGF* encodes for a neuroprotective matrix metalloproteinase, and may be related to inflammatory demyelization in the central and peripheral nervous system (Hartung et al. 2000).

The *Histone deacetylase 3* (*HDAC3*) [OMIM 605166] on chromosome 5q31.3 showed significant association with executive functioning (Stroop Interference Score) in the AF subsample. HDAC3 is one of the key regulators in cell proliferation, and its inactivation results in delay of the cell cycle, DNA damage, and apoptosis (Bhaskara et al. 2008).

The *Protein phosphatase 2, regulatory subunit B, beta isoform* (*PPP2R2B*) [OMIM 604325] on chromosome 5q32 showed significant association with visual working memory in the AF subsample. *PPP2R2B* regulates protein phosphatase 2A which plays a role in various tauopathies. The CAG repeat length in *PPP2R2B* has shown association with inherited ataxia (Holmes et al. 1999), and Alzheimer's disease (Chen et al. 2009). However, any association with schizophrenia is lacking (Laurent et al. 2003).

The *Casein kinase 1 alpha 1* (*CSNK1A1*) [OMIM 600505] on chromosome 5q33.1 showed significant association with processing speed in the whole sample (ALL). *CSNK1A1* encodes for a protein kinase which is associated with neurodegenerative disorders (Knippschild et al. 2005), and suggestively with a rat model of ADHD (DasBanerjee et al. 2008).

The *Calcium/calmodulin-dependent protein kinase II alpha* (*CAMK2A*) [OMIM 114078] on chromosome 5q33.2 showed significant association with verbal attention in the whole sample. *CAMK2A* encodes for a protein kinase which shows increased expression in major depression (Tochigi et al. 2008) and is involved in adaptation to stress (Muller et al. 2003). In mice, *CAMK2A* is downregulated under chronic mild stress (Orsetti et al. 2008).

The *Glutamate receptor, ionotropic, AMPA 1* (*GRIA1*) [OMIM 138248] on chromosome 5q33.2 showed multiple suggestive associations with the severity of positive symptoms of schizophrenia, and the association was stronger among the IS subsample. *GRIA1* encodes for a glutamate receptor, and its elevated mRNA levels are detected in brains of schizophrenia patients (O'Connor et al. 2007). Furthermore, *GRIA1* has been associated with schizophrenia (Magri et al. 2006), and psychotic bipolar disorder (Kerner et al. 2009).

Table 15. Association results on chromosome 5q31.1-5q33.3. Beta values are for the untransformed trait. Only P-values below the suggestive threshold are shown, and P-values below the significant threshold are shown in bold.

SNP	Gene	Allele[a]	P-value	Trait	Beta	Sample
rs3756295	*SPEC2*	G	0.0004	ViAt	-0.50	CTRL
rs2079103	***RAD50***	**A**	**0.00006**	**AOO**	**-3.53**	**AF**
rs269783	***PURA***	**G**	**0.00005**	**SDe**	**-1.17**	**AF**
		A	0.0002	VeAt	-0.47	IS
rs155946	*PURA*	A	0.0002	SDe	-0.99	AF
rs7268	***HBEGF***	**A**	**0.000006**	**SDe**	**-1.07**	**AF**
rs2237078	*HBEGF*	C	0.0003	SDe	-0.94	AF
rs3844598	***HDAC3***	**A**	**0.00002**	**S**	**12.18**	**AF**
rs11742646	*HDAC3*	G	0.0004	S	10.78	AF
rs251041	*HDAC3*	C	0.0004	S	11.57	AF
rs6580194	*CENTD3*	T	0.0004	S	11.23	AF
rs4912905	*NR3C1*	G	0.0003	EF	26.65	AF
rs7713438	*PPP2R2B*	G	0.0005	S	8.89	ALL
rs11952689	***PPP2R2B***	**C**	**0.00002**	**ViWM**	**-1.12**	**AF**
rs7721529	***CSNK1A1***	**A**	**0.00004**	**PS**	**-1.74**	**ALL**
rs1947582	*CSNK1A1*	A	0.0003	PS	-1.45	ALL
		A	0.0002	PS	-3.07	AF
rs17712679	***CAMK2A***	**A**	**0.00001**	**VeAt**	**-0.42**	**ALL**
		A	**0.00003**	**VeAt**	**-0.45**	**IS**
rs12515622	*GRIA1*	G	0.0005	ViAt	-0.37	ALL
rs10057063	*GRIA1*	A	0.00008	SAPS	9.62	IS
rs2216649	*GRIA1*	C	0.0002	SAPS	6.31	ALL
		C	0.0001	SAPS	9.18	IS
rs12189362	*GRIA1*	G	0.00008	SAPS	8.61	IS

[a]Allele for which the effect is shown

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; A, adenine; C, cytosine; G, guanine; T, thymine; ALL, whole sample; IS, internal isolate; AF, rest of Finland (outside the isolate); CTRL, control sample; for gene name abbreviations, refer to the Abbreviations chapter; for trait abbreviations, refer to Table 9

6.3.4 Conclusions

The studied chromosomal regions were selected based on our previous genome-wide linkage analyses (Paunio et al. 2001, Paunio et al. 2004). Although the 2q and 5q loci have proved to be relevant in international meta-analyses as well (Lewis et al. 2003, Ng et al. 2009) and all the regions were initially screened for all the known genes, the altogether 1511 selected SNPs failed to reveal any significant associations with the clinical diagnostic categories of schizophrenia and related disorders. A number of regional and functional candidate genes showed, however, association with both the clinical symptoms of schizophrenia as well as for cognitive features considered relevant schizophrenia endophenotypes.

Of the highlighted genes, of special interest is *ERBB4*, showing association with the severity of positive symptoms and impairments in traits related to verbal attention and

verbal ability, since *ERBB4* has been previously associated with schizophrenia and verbal working memory (Silberberg et al. 2006, Nicodemus et al. 2006). Similarly, *GRIA1* showed association with the severity of positive symptoms, and has been previously associated with schizophrenia (Magri et al. 2006). Of the other genes, *EPHA4* showed suggestive association with schizophrenia spectrum disorders and several clinical disorder features, and *EPHA5* with several verbal learning and memory traits, and interestingly, these ephrin receptors not only play critical roles in the development of CNS but also in modifying synaptic connections in mature brain, supporting their plausible roles in the pathogenesis of schizophrenia (Murai et al. 2002).

Our material involving only the known functionally relevant genes from the linked regions leaves a possibility for unintentional exclusion of some potentially relevant genes or regulatory regions. However, the accurate location of linkage signals is prone to fluctuation in complex disorders with equivocal inheritance (Roberts et al. 1999, Altmuller et al. 2001), and this creates a possible caveat for all candidate gene analyses based on genome-wide screens. In any case, any single regional gene is unlikely to solely explain our previous linkage results. Instead, those linkage signals may represent a net effect from multiple variants associated with a diverse spectrum of features affecting distinct disorder domains (Roberts et al. 1999, Altmuller et al. 2001). These results may therefore be in line with the observation of a net influence of detected variants on the overall disorder liability (Toulopoulou et al. 2007).

The partly distinct associations detected in our subsamples fit both the known genetic substructure of the Finnish population (Salmela et al. 2008, Jakkula et al. 2008), and the previous evidence of different risk variants and/or differences in their frequencies affecting the disorder liability in and outside the isolate (Hovatta et al. 1997, Paunio et al. 2001). Additionally, the distinct associations detected among schizophrenia families and controls may represent divergent effects in the presence or absence of the disorder load, that is, in conjunction with other variants directly increasing the liability (Fanous et al. 2001, Fanous et al. 2005). Overall, that any single high-risk determinant remained absent in our study is also in line with the recent genome-wide studies showing the role for number of variants with small individual effects (O'Donovan et al. 2008, International Schizophrenia Consortium et al. 2009, Shi et al. 2009, Stefansson et al. 2009). The risk for false negative results, however, cannot be ruled out, although a number of detected signals still passed our threshold for significant association. Furthermore, these thresholds were calculated by using Bonferroni correction, which in genetic studies is rather conservative than liberal (Balding 2006). The Bonferroni correction was selected, however, due to its wide use in the field of statistics, and since no "golden standard" exists yet regarding the multiple testing correction in genetic studies.

These results warrant further studies on the highlighted genes to evaluate their plausible roles in the pathogenesis of schizophrenia and, additionally, of the related psychotic disorders. Overall, these results revealing several significant associations for different genes encourage the use of intermediate phenotypes in the search of predisposing variants for schizophrenia. In addition, these results highlight the difficulties in discovering the key genetic players behind the linkage signals, making future studies on these associated gene variants essential, or rather, the combined roles of these variants.

7 Concluding remarks

This study provides evidence for the plausible role of several candidate genes in the genetic liability of schizophrenia and related psychotic disorders. The effective and long-term collaboration between the number of experts in medical genetics and clinical psychiatry, as well as achievements of the Finnish public health care, has made this study possible.

The heterogeneity in the genetic background of schizophrenia favors the use of as large and well-characterized sample sets as possible, as well as replication efforts to help in the estimation of the impact of identified risk variants. Our study I represents a straightforward attempt to try to replicate the previous positive associations between schizophrenia and candidate genes *DTNBP1*, *NRG1*, and *AKT1*. Despite our reasonably large sample set with reliable clinical assessment, any significant associations with these genes remained absent. While the relatively low number of analyzed markers, especially for *NRG1*, leaves the possibility of missing some rare risk-increasing variants, our results may indeed reflect the minor role of these genes in the genetic liability of schizophrenia in Finland.

The phenotypic heterogeneity within the core diagnoses of psychiatric disorders, especially for schizophrenia, challenges the traditional genetic analyses which use solely the clinical diagnosis as trait information. Although fine-tuning the diagnostic criteria may provide some help, further dissecting the disorder background with trait components, clinical features, and endophenotypic measurements may allow more powerful detection of variants affecting the individual disorder domains. As shown for *RELN* in parts II and III of this study, the use of additional phenotypic information may elucidate intriguing results. Although the identified risk allele in *RELN* may reflect the effects of some other causative variant, our results support the involvement of *RELN* in schizophrenia liability and especially its role as a modifier of disorder-related characteristics.

The successful utilization of extensive phenotypic information in our prior analyses encouraged also the mapping of potential candidate genes on the chromosomes 2q, 4q, and 5q. These loci were highlighted in our previous genome-wide linkage analyses, and importantly, also partly in international schizophrenia linkage meta-analyses. Interestingly, several genes showed promising associations with traits supposedly representing different disorder domains. From the wide spectrum of genes, of special interest are *ERBB4*, which showed association with the same verbal attention trait as in a previous analysis, and *GRIA1*, which showed association with the severity of positive symptoms of schizophrenia, in line with a previous association with schizophrenia liability.

When this study began, the era of linkage analyses was already coming to its end. Tens of genome-wide and numerous smaller linkage scans of schizophrenia had pointed to nearly all chromosomal regions, providing no conclusive evindence for any major genetic determinant. The field of psychiatric genetics had already started shifting towards association analyses, for which also the first genome-wide genotyping technologies were soon to be introduced. Although the wide range of association studies based on individual candidate genes have provided evidence for the plausible involvement of several genes, the results have been notably diverse for many of them. So

far, the results from the recent large genome-wide association analyses have ensured that there really are no low-hanging fruits when it comes to genetics of schizophrenia.

The novelty value of this study is largely based on the versatile use of different measurable characteristics in addition to clinical diagnostic information. These characteristics, such as endophenotypes, the intermediate factors between clinical phenotype and genotype, as well as clinical measurements, may mediate or reflect the risk to catch the disorder. The use of endophenotypes and other intermediate factors is still reasonably new in the field of psychiatric genetics, although the concept itself has been itroduced already decades ago. The toilsomeness and expensiveness of the required individual testing are supposedly the principal factors limiting the more extensive use of, for instance, these different cognitive measurements. Here, the far-sighted investments of the National Institute for Health and Welfare in these assessments now bear fruit.

To conclude, this study provides evidence for the involvement of *RELN* in the pathogenesis of schizophrenia, and in addition, supports the roles of several other candidate genes located on known schizophrenia loci. In general, this study supports the view that only detailed phenotypic data may allow for identification of all the risk variants behind schizophrenia, and supports the use of endophenotypes in studies on schizophrenia genetics. Although the results presented here add small individual pieces to the genetic puzzle of this disorder, these studies also reveal the challenges of schizophrenia genetics. Even in the Finnish population, several genes, probably in many different combinations, are likely to account for the risk of schizophrenia and the related phenotypic features.

8 Future prospects

Traditionally, schizophrenia and the psychiatric disorders overall have been highly stigmatizing for the affected individuals as well as their close relatives, especially for the parents of the patients. The accumulation of knowledge of the factors predisposing to schizophrenia help, in addition to diagnostic and treatment procedures, to relieve the negative social load on the patients and their family members. As an example of research influence, the old view on bad parenting, especially bad mothering, as the main cause of the disorder has been disproved already decades ago.

Despite the high heritability of schizophrenia and intensive research efforts during the past decades, any major genetic determinant of liability has remained unidentified. This has lead to the present hypothesis of multiple genetic factors with usually small individual effects constituting the liability in conjunction with the environmental factors which may, at least in part, "discharge the weapon armed by the genes". Furthermore, different genetic variants may affect different disorder domains, for instance, cognitive functions. This challenges the possibilities of sole diagnosis-based variant detection. Instead, more detailed analysis of different disorder characteristics may help in the gene identification, especially as the combinations of the variants rather than the individual variants themselves may determine the liability. Furthermore, since the genes are unlikely to follow any diagnostic boundaries of psychiatric disorders, same variants may appear in the background of diagnostically distinct entities.

In the present era of large-scale genotyping and, in the near future, genome-wide sequencing, the amount of information in the analyses will literally explode. This may challenge the analysis techniques, as the line between the false and real signals may remain unclear, especially due to problems related to multiple testing. Evidently, the analyses will also be broadened more and more beyond the traditional straightforward analysis between the variants and traits to include gene-gene interactions, gene-environment interactions, and pathways. Additionally, different epigenetic factors need to be taken into account, and the growing knowledge of these will undoubtedly reflect the study designs. The accuracy of the utilized phenotypic information is of concern as well. That the psychiatric diagnoses are based on symptoms, not objective laboratory tests, will be still a concern in the foreseeable future. Supposedly the use of different intermediate phenotypes and factors will broaden, our study being among those supporting this development. The challenges in collection of these data will, however, still require extensive organization in addition to adequate financial investments. Also in contrast to genotyping, the gathering of these data will supposedly still be as time-consuming as it is today.

The results presented here encourage further evaluation of the highlighted genes, especially *RELN*, in the pathogenesis of schizophrenia. Multiple-line evidence suggests *RELN* involvement in several psychiatric and neurodevelopmental disorders. Although *RELN* may play a role in different psychiatric conditions, any targeted diagnostic or treatment options for schizophrenia are unlikely to be developed in the near future. This is not only due to incomplete information on *RELN* itself, however, but also due to limitations in treatment options. The present pharmacological intervention in psychiatric disorders is based on drugs affecting broadly the different neurotransmitter systems of the central nervous system. To utilize the more specific knowledge on disorder

mechanisms also more targeted treatment technologies need to be developed. The progress in, for instance, cancer treatment options is encouraging, however, although the same methodology may not be adapted to psychiatry.

Whether low-cost sequencing of the whole genome and assessment of the role of several individual risk variants will allow better diagnostics and treatment of schizophrenia remains to be seen. The active research in psychiatric genetics, and also in clinical psychiatry, will undoubtedly reveal novel data behind schizophrenia and its liability during the next decades. Translational research combining the wide spectrum of clinical data and basic research will be essential to develop even individualized therapeutic options. Only science itself can show how distant that future may be.

9 Acknowledgments

This study was carried out in Public Health Genomics Unit, National Institute for Health and Welfare THL (formerly Department of Molecular Medicine, National Public Health Institute KTL), and Institute for Molecular Medicine Finland FIMM, Nordic EMBL Partnership for Molecular Medicine. The Director General of THL Pekka Puska, the Director of Public Health Genomics Unit Anu Jalanko, and the Director of FIMM Olli Kallioniemi are acknowledged for providing excellent research facilities.

The essential collaboration of all the participating individuals and their families, and their supportive and understanding attitude towards our research is highly appreciated. Furthermore, all the field workers and clinicians who have participated in the sample collection and diagnostic assessment are greatly acknowledged for their fundamental work. It is needless to state that without all these individuals this study would not have been possible in the first place.

I have had the privilege to work under supervision of Academician Leena Peltonen-Palotie, who really gives a meaning to term "world-class scientist". Leena's expertise, energy and enthusiasm cannot be but admired. Although being definitely one of the leading geneticists in the world, Leena has kept the hierarchy low in our research group and her office door literally open at all times for us junior researchers to pop in whenever we have needed the guidance of her wisdom and vision. On the other hand, Leena has also given us much responsibility on our own, letting us to feel being trusted and respected. During the years, Leena has established a positive and high-aiming atmosphere in our group, which has driven it to better and better achievements.

I wish to deeply thank my other supervisor Anu Loukola, who has been my hands-on teacher in the laboratory, tireless reviewer of my manuscripts, and supporter on my way to become a scientist myself. In addition to all her efforts in guiding my thesis project, Anu has played a notable part in helping me broaden my projects beyond schizophrenia.

I also want to express my deepest gratitude for Adjunct Professor Tiina Paunio, one of the key members of our research group. If regulations only had allowed, Tiina would have justifiably been included as my third supervisor, her kind support having been way beyond regular collaboration. Tiina's knowledge in psychiatry and psychiatric genetics have been invaluable for my thesis project. Furthermore, Tiina has expressed her trust and support by taking me into her other international collaboration projects as well.

I am grateful for Professors Matti Isohanni and Jari Tiihonen, leading Finnish experts in the field of psychiatry, for reviewing my thesis and giving valuable criticism and excellent remarks. I am thankful for Professor Markus M. Nöthen, the greatly appreciated scientist, for accepting to be the official opponent in my thesis defence.

I wish to express my appreciation and gratitude to all the co-authors of my publications, especially Professor Jouko Lönnqvist and the Adjunct Professors Annamari Tuulio-Henriksson, Jaana Suvisaari, Teppo Varilo, Samuli Ripatti, and Timo Partonen for their substantial contributions. Annamari and Jaana have been nothing but essential in all my study sample and phenotype-related issues, and while the occasions when I have needed their help have been numerous, they have always had the time for me and my questions. Teppo is the person behind the valuable genealogical information from our study sample, and in addition to being excellent scientist, he has also been excellent company

in and outside the laboratory, through which I have really understood the whole meaning of the word "colleaque". Samuli is thanked for his valuable help in the issues related to statistical analysis, and his ability to explain these aspects in an understandable way. Besides being an important collaborator, Timo has also been a member of my thesis committee, for which he is highly acknowledged.

The other members of my thesis committee, Academy Professor Lauri Aaltonen, and Professor Eero Castrén are also acknowledged for their valuable support. I am grateful for having these three highly respected top scientists by my side all these years.

Further thanks regarding the statistical analysis issues go to Professor Joseph Terwilliger, Adjunct Professor Markus Perola, Tero Hiekkalinna, and Ida Surakka, whose help has been extremely precious. The technical assistance by Kaisa Silander and Pekka Ellonen in genotyping has not only been supportive, it has been invaluable. The same applies to especially Elli Kempas and Liisa Arala, whose professional work in the laboratory has formed the backbone for this study, as well as to Minttu Jussila, Siv Knaappila, Sisko Lietola, Anne Nyberg, Minna Suvela, Outi Törnwall, and Anne Vikman, whose technical assistance is highly appreciated. Marjut Grainger is thanked for her effective work in sample and phenotype data handling.

I am most grateful to Sari Kivikko for her kind and effective help in various and several practical issues during my thesis project. Sari has really been "the right hand" for the whole research group of ours. Also Mika Kivimäki, Tuija Koski, Liisa Penttilä, and Sanna Tossavainen have been most helpful in a variety of practical matters. Marjo Kestilä is especially thanked for her practical help and support in my thesis finalization.

I want to thank the highly talented people in our research group for all their help and good company in and outside the laboratory. Jesper is thanked for his guidance when I first started the project, and overall for his knowledge and support along the way. Joni and Olli are especially thanked for being superior company and for their optimistic attitude towards both science and life in general. Ansku, Marine, and William are thanked for being great officemates, and William additionally for always being one to rely on in various scientific issues. Annika, Annu, Antti, Antti-Pekka, Anu K, Emília, Emma N, Emma P, Emmi, Eveliina, Hanski, Heidi, Helena, Henna, Iiris, Jaana W, Jarkko, Jenni, Johannes, Jonas, Jonna, Jussi, Kaisu, Karola, Kati, Katri, Laura, Liisa, Mari, Marika, Markus L, Mervi, Mikko, Nan, Peter, Pia H, Pia S, Pirkka-Pekka, Sampo, Suvi, Tero Y, Tiia, Tintti, and Virpi, in addition to those I probably forgot to mention, are thanked for being the best possible peer group. Of the colleaques outside our research group I would like to thank especially Verneri for his brilliant scientific mind and endless optimism.

Despite the continuing attempt to replace social life with work I have somehow managed to keep many invaluable friends. Thank you for all the good times we have had together. You all are beyond words. Spesifically, I would like to thank all the fellow instrument owners in Retuperän WBK. See you next Tuesday. And especially, I would like to thank the Fire Chief Leevi Letkutsalo since it is always good to thank the Fire Chief.

My warmest gratitude goes to my parents Irmeli and Erkki, for their love, encouragement and endless support for my education and activities throughout my life. My sister Reetta and brother Lauri have provided me with enjoyable company and lively moments. In addition, my mother-in-law Eeva-Kaisa and father-in-law Martti deserve thank for their care and support.

Finally, I want to thank the most important person in my life, my beloved wife Satu. We have shared so much, profession, friends, values, life itself. Together we have studied medicine, worked as physicians, attended scientific conferences, played in orchestras, hiked in mountains, flown to the other side of the globe. I wouldn't be here without you. You are the light of my life.

This study was financially supported by the Academy of Finland (Center of Excellence in Complex Disease Genetics), Biocentrum Helsinki, and the European Commission (LSHM-CT-2006-037761). Juho Wedenoja was financially supported by Helsinki Biomedical Graduate School, as well as personal grants from (in alphabetical order) Biomedicum Helsinki Foundation, Emil Aaltonen Foundation, Finnish Cultural Foundation, Finnish Medical Foundation, Jalmari and Rauha Ahokas Foundation, Orion-Farmos Research Foundation, and Psychiatry Research Foundation.

Helsinki, 13 January 2010

Juho Wedenoja

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