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MOLECULAR GENETICS OF SCHIZOPHRENIA AND COMORBID AND RELATED TRAITS

by

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Academic Dissertation

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"Prudens quaestio dimidium scientiae."
("Half of science is asking the right questions.")
- Roger Bacon (1214-94)

"If the brain were so simple we could understand it,
we would be so simple we couldn't"
- Lyall Watson (1939-)

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, referred to in the text by roman numerals. In addition, some unpublished data are presented.

I. Ekelund J, Lichtermann D, Hovatta I, Ellonen P, Suvisaari J, Terwilliger JD, Juvonen H, Varilo T, Arajärvi R, Kokko-Sahin M-L, Lönnqvist J, Peltonen L (2000) Genome-wide scan for schizophrenia in the Finnish population: evidence for a locus on chromosome 7q22. Human Molecular Genetics 9(7):1049-1057

II. Paunio T, Ekelund J, Varilo T, Parker A, Hovatta I, Turunen J, Rinard K, Foti A, Terwilliger JD, Suvisaari J, Arajärvi R, Partonen T, Juvonen H, Lönnqvist J, Meyer J and Peltonen L (2001) Genome-wide scan in the nationwide study sample of schizophrenia families in Finland. Human Molecular Genetics (in press)

III. Ekelund J, Hovatta I, Parker A, Paunio T, Varilo T, Martin R, Suhonen J, Ellonen P, Chan G, Sinsheimer JS, Sobel E, Juvonen H, Arajärvi R, Partonen T, Suvisaari J, Lönnqvist J, Meyer J and Peltonen L (2001) Chromosome 1 Loci in Finnish Schizophrenia Families. Human Molecular Genetics 10(15):1611-1617

IV. Lichtermann D*, Ekelund J*, Pukkala E, Tanskanen A, Lönnqvist J (2001) Incidence of Cancer Among Persons with Schizophrenia and their Relatives. Archives of General Psychiatry 58(6):573-578

V. Ekelund J, Lichtermann D, Järvelin M-R, Peltonen L (1999) Association between Novelty Seeking and the type 4 dopamine receptor gene in a large Finnish cohort sample. American Journal of Psychiatry 156(9):1453-1455

* These authors contributed equally.

ABBREVIATIONS

ASP	affected sibpair
bp	base pair
cM	centimorgan
CI	confidence interval
CT	computed tomography
DNA	deoxyribonucleic acid
DSM	Diagnostic and statistical manual of mental disorders
DZ	dizygotic
EEG	electro-encephalogram
GDB	Genome Database
h^2	heritability
HHRR	haplotype-based haplotype relative risk
IBD	identical by descent
IBS	identical by state
ICD	International classification of diseases
LC	liability class
LD	linkage disequilibrium
Lod	logarithm of odds
MLS	maximum likelihood score
MRI	magnetic resonance imaging
MZ	monozygotic
n	number
OR	odds ratio
PCR	polymerase chain reaction
PET	positron emission tomography
QTL	quantitative trait locus
RFLP	restriction fragment length polymorphism
RH	radiation hybrid
SNP	single nucleotide polymorphism
SPECT	single-photon computed tomography
TCI	temperament and character inventory
TDT	transmission/disequilibrium test
VNTR	variable number of tandem repeat
WHO	World Health Organization
Z_{max}	maximum lod score

ABSTRACT

Molecular genetic methods have successfully been employed in research on rare, monogenic diseases. Spurred by this success, researchers have employed the same methods for common complex traits like cardiovascular, immunological and psychiatric disorders. In this thesis various strategic and statistical methods were used to study the genetic component of schizophrenia, its comorbidity and related traits.

We collected DNA samples from a large number of families affected with schizophrenia. These samples were used for genetic studies of the etiology of schizophrenia. We performed a genome-wide scan in a sib-pair collection from the isolated Finnish population. The strongest evidence for linkage was obtained on chromosome 7q22. The next strongest evidence for linkage was seen on chromosome 1q42, in the vicinity of the locus implicated in another genome-wide scan for schizophrenia performed in Finnish families from a regional sub-isolate. To further position the region of interest we genotyped a dense marker map on chromosome 1q. A total of 147 polymorphic markers on chromosome 1 were included in this part of the study. The best evidence for linkage was seen for a marker situated within a promising candidate gene for schizophrenia (DISC1). Finally, we performed a third genome-wide scan using a larger study sample than in the two previous studies. Two new regions showing evidence for linkage were identified, one on chromosome 2 and another on chromosome 5. The locus on chromosome 2 has not previously been reported to be linked to schizophrenia, while the finding on chromosome 5 represents a strong replication of several other studies reporting evidence for linkage to this region.

In another line of studies we investigated comorbid traits of schizophrenia. First we monitored the age- and calendar-year-corrected incidence of cancer in schizophrenic patients. This study was initiated because of previous reports of lower cancer incidence in schizophrenic populations, a highly counter-intuitive finding given the high rates of smoking among schizophrenia sufferers. We were able to achieve virtually complete ascertainment of both schizophrenia and cancer thanks to the systematic registration of both diseases in Finland. In addition, it was possible to investigate the cancer incidence in the first-degree relatives of the schizophrenic patients, since they could be identified from the population register. They share on average half of their genes with patients, but are not exposed to the disease specific environmental factors like long hospitalization. We found a slightly increased incidence of cancer in the schizophrenic patients, in contradiction of the earlier evidence. We also found a moderately decreased risk of cancer in the first-degree relatives of the schizophrenic patients. The interpretation of this finding remains unclear, both environmental and genetic protective factors being conceivable.

Second, we tried to replicate a reported association between dopamine receptor 4 (DRD4) and the human personality trait of Novelty Seeking (NS). NS is a normally distributed trait in the population with considerable heritability. Identification of variants in genes that affect normal personality could give important information on the etiology of psychopathology. We did find an association between NS and DRD4 by studying individuals with extremely low and extremely NS high scores. Interestingly, high NS score was associated with a different variant of the DRD4 gene in our sample than in the original reports. This probably indicates that the studied variant in itself would not influence NS, but rather some other variant within the same gene, or in a gene situated very close to DRD4 on chromosome 11p.

INTRODUCTION

Schizophrenia is a severe mental disorder affecting approximately one percent of the population worldwide. It affects all aspects of cognition, perception, affect and behavior, and is therefore one of the most disabling and emotionally devastating illnesses known to man. The etiology of schizophrenia is unknown, but based on family, twin and adoption studies, genetic liability plays an important role. However, the genetic factors underlying the disorder, as well as the environmental stressors contributing to the onset of clinical symptoms, remain largely unknown.

Due to its relative genetic isolation, Finland is likely to offer some advantages when searching for genes underlying complex diseases such as schizophrenia. Since Finland first became home to a small group of settlers, the population has expanded to its present size of slightly over five million. It can therefore be assumed that fewer alleles of predisposing genes underlie any disorder than in more mixed populations. This increases the potential to localize susceptibility genes by linkage and association analysis in study samples from this isolated population. This advantage is even more pronounced in sub-isolates within the population with multiple bottlenecks in the population history. We have therefore systematically collected samples from one such inbred sub-population. To fully understand the concept of schizophrenia it is also important to study its comorbidity. Traits that occur more or less frequently among persons suffering from schizophrenia can shed some light on the etiology and risk factors for either or both of the traits. Thus, we have also investigated associated traits of schizophrenia using a unique population cohort collected in Finland and the registers available in this population.

REVIEW OF THE LITERATURE

1 Schizophrenia

1.1 Diagnosis

In 1896 Emil Kraepelin introduced the term "dementia precox" for the large body of patients suffering from a deteriorating mental disorder with an early onset (Kraepelin 1919). The term dementia precox emphasizes the cognitive facet (dementia) and the early onset (precox) of the disorder. The diagnosis of dementia precox was characterized by a deteriorating course and clinical symptoms of hallucinations and delusions. Kraepelin distinguished the patients suffering from dementia precox from those suffering from manic-depressive psychosis or paranoia. The concept of schizophrenia was first used by Eugen Bleuler in 1911 (Bleuler 1950). The term was coined to signify a schism between thought, emotion and behavior, *not* split personality (a rare disorder termed dissociative identity disorder in modern disease classifications) as the term is often misinterpreted by the lay public. Bleuler divided the symptoms into primary and secondary symptoms. The primary symptoms were Associational disturbances, Affective disturbances, Autism and Ambivalence (the four As, Table 1), and the secondary symptoms were hallucinations and delusions. The most fundamental difference between Kraepelin's and Bleuler's concepts was the deteriorating course of the illness, fundamental in Kraepelin's view of the disorder, but not a necessary prerequisite according to Bleuler. The modern diagnostic systems have clearly moved towards Kraepelin's view, while Bleuler's term "schizophrenia" has become internationally accepted.

Presently, the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) published by the American Psychiatric Association (1994) and the International Classification of Diseases, 10th edition (ICD-10) published by the World Health Organization (1994) co-exist as diagnostic systems in clinical work and research in the industrialized world. In the European countries, ICD-10 is the official system for clinical diagnoses, while DSM-IV is dominant in research settings. According to DSM-IV at least two of the following have to be present for a significant portion of time during a 1-month period (or less if successfully treated): 1. delusions, 2. hallucinations, 3. disorganized speech, 4. grossly disorganized or catatonic behavior, 5. negative symptoms, i.e. affective flattening, alogia or avolition. In addition to these characteristic symptoms, signs of the disorder have to persist for at least six months and have a marked negative effect on social and/or occupational functioning. It must also be confirmed that the symptoms are not due to other disorders, like

Table 1. Fundamental symptoms of schizophrenia according to Bleuler (1950)

1. Association
- Lack of purpose or goal in the speech; poverty of ideas
- Stereotypy; echolalia
- Thought blocking
- Pressure of thought; clang associations

2. Affectivity
- Lack of depth to the affect; restricted affect
- Lack of consistency of affective manifestation
- Inappropriate of blunted affect

3. Attention
- Lack of selectivity of attention; impaired active attention

4. Ambivalence
- Affective ambivalence: the same concept is accompanied simultaneously by pleasant and unpleasant feelings
- Ambivalence of will: the patient wishes and does not wish the same thing at the same time
- Intellectual ambivalence: the patient expresses contradictory thoughts in the same sentence

5. Autism

schizoaffective disorder and mood disorders, or to the effect of some substance or general medical condition (Table 2). According to ICD-10, the general criteria for schizophrenia require one of the following to be present for most of the time during a 1-month period: 1. thought echo, insertion, withdrawal or broadcasting, 2. some specific delusions referred to body movements, specific thoughts or sensations, or other persistent, culturally inappropriate and completely impossible delusions. 3. hallucinatory voices. Alternatively, two of the following must be present most of the time for a 1-month period: 1. any persistent hallucinations, 2. neologisms, breaks, or interpolations in the train of thought, resulting in incoherence or irrelevant speech, 3. catatonic behavior, 4. negative symptoms such as marked apathy, paucity of speech, and blunting or incongruity of emotional responses (Table 3).

Table 2. Diagnostic criteria for schizophrenia according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders (APA 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 is successfully treated):

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

Note: 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 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, Manic, or Mixed Episodes have occurred concurrently with the active-phase symptoms; or (2) of 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 the substance (e.g., a drug of abuse, a medication) or a general medical condition.

F. *Relationship to 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).

Table 3. Diagnostic criteria for schizophrenia according to the ICD-10 Classification of Mental and Behavioural Disorders (WHO 1994).

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

(1) At least one of the following must be present:

(a) thought echo, thought insertion or withdrawal, or thought broadcasting;
(b) delusions of control, influence, or passivity, clearly referred to body or limb movements or specific thoughts, actions, or sensation; delusional perception;
(c) hallucinatory voices giving a running commentary on the patient's behaviour, or discussing the patient among themselves, or other types of hallucinatory voices coming from some part of the body;
(d) persistent delusions of other kinds that are culturally inappropriate and completely impossible (e.g. being able to control the weather, or being in communication with aliens from another world)

(2) Or at least two of the following:

(a) persistent hallucinations in any modality, when occuring every day for at least 1 month, when accompanied by delusions (which may be fleeting or half-formed) without clear affective content, or when accompanied by persistent over-valued ideas;
(b) neologisms, breaks, or interpolations in the train of thought, resulting i incoherence or irrelevant speech;
(c) catatonic behaviour, such as excitement, posturing or waxy flexibility, negativism, mutism, and stupor;
(d) "negative" symptoms, such as marked apathy, paucity of speech, and blunting or incongruity of emotional responses (it must be clear that these are not due to depression or neuroleptic medication).

G2. Most commonly used exclusion clauses

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

(2) The disorder is not attributable to organic brain disease (in the sense of F00-F09), or to alcohol- or drug-related intoxication (F1x.0), dependence (F1x.2), or withdrawal (F1x.3 and F1x.4).

As described above, schizophrenia was demarcated as a specific diagnostic entity at the end of the 19th century. However, it is likely that the disorder has existed in historic times. In the literature, accounts of persons "going mad" exist already in the classical tragedies. According to the American psychiatrist Nancy Andreasen (Andreasen 2000) e.g. Shakespeare depicted quite accurately the symptoms of schizophrenia in characters such as Ophelia in Hamlet and 'Poor Tom', son of Gloucester, in King Lear. According to other theoreticians, however, the first accurate description of what we today call schizophrenia occurred only in 1809, simultaneously in France and England (Gottesman 1991). John Haslam (1764-1844), superintendent of the Bethlem Hospital in London published the following description of unmistakable schizophrenia:

" There is a form of insanity which occurs in young persons; and as far as these cases have been the subject of my observation, they have been more frequently noticed in females. Those whom I have seen, have been distinguished by prompt capacity and lively disposition; and in general have become the favorites of parents and tutors, by their facility in acquiring knowledge, and by a prematurity of attainment. This disorder commences, about or shortly after, the period of menstruation, and in many instances has been unconnected with hereditary taint, as far as could be ascertained by minute inquiry. The attack is almost imperceptible; some months usually elapse before it becomes the subject of particular notice; and fond relatives are frequently deceived by the hope that it is only an abatement of excessive vivacity, conducting to a prudent reserve, and steadiness of character. A degree of apparent thoughtfulness and inactivity precede, together with a diminution of the ordinary curiosity, concerning that which is passing before them; and they therefore neglect those objects and pursuits which formerly proved courses of delight and instruction. The sensibility appears to be considerably blunted: they do not bear the same affection towards their parents and relations: they become unfeeling to kindness, and careless of reproof. To their companions they show a cold civility, but take no interest whatever in their concerns… Thus in the interval between puberty and manhood, I have painfully witnessed this hopeless and degrading change, which in a short time has transformed the most promising and vigorous intellect to a slavering and bloated idiot." (Haslam 1809/1976)

In this description of a condition very much reminiscent of schizophrenia, we can see two claims that have later been disproved: The higher prevalence in females and the low heritability. Despite these shortcomings, it is clear that the above description is referring to schizophrenia, and we can conclude that schizophrenia has been present at least from the beginning of the 19th century, and probably much earlier.

1.2 Epidemiology

Epidemiologic findings in schizophrenia can guide our search for the ultimate goal - understanding the etiology and pathophysiology of schizophrenia - especially some consistent and quite simple findings:

The prevalence of schizophrenia has been reported to be 1-1.5% in most studied populations (Sartorius et al. 1986; Torrey 1987). It must be noted that since schizophrenia is normally a lifetime disorder, the lifetime prevalence and point prevalence are essentially identical. Certain important populations in which the prevalence deviates significantly from this figure exist. One example is an internal isolate in Finland in which the lifetime prevalence is 2.2% and the age-corrected lifetime risk is 3.2% (Hovatta et al. 1997). In fact, up to 50-fold differences in the prevalence of schizophrenia have been observed (from 0.3 to 17/1000) (Torrey 1987). However, the fact remains that schizophrenia exists at approximately the same rate in geographic regions with very different culture, degree of industrialization, climate etc. This may suggest that these factors cannot play a decisive role in the etiology of schizophrenia. From a genetic perspective, this finding suggests either that A) the mutations increasing the susceptibility to schizophrenia are very old, i.e. from the time before the proposed emigration of the human species out of Africa some 35,000 to 89,000 years ago (Underhill et al. 2000), or B) de novo mutations in the genes affecting susceptibility for schizophrenia have occurred at similar rates in all populations worldwide, with the important exception of some young internal isolates showing effects of founder mutations and genetic drift.

As was described previously, there remains some controversy over whether accurate descriptions of schizophrenia exist before the beginning of the 19th century, and what its possible prevalence might have been in historical times. According to some theories, schizophrenia has probably existed as long as the human species. This assumption is based on the necessity of lateralization of the brain for human language capacity, and the deficient brain lateralization seen in schizophrenic subjects (DeLisi et al. 1997). However, this subject remains speculative and no certainty about the possible existence of schizophrenia and its prevalence is likely to be obtained.

Schizophrenia is equally prevalent in men and women; at least there are no consistently replicated findings to the contrary. Some studies have found an excess of males (Hovatta et al. 1997; Kendler and Walsh 1995), while some have found no consistent difference, especially when the diagnostic criteria are broadened (Jablensky et al. 1992). Men do, on average, have an earlier onset and a more severe course of the disorder (Castle et al. 1995). The peak age of onset for men is in the early twenties, and for women in the mid-late twenties with a second peak in the years around menopause (Castle et al. 1995; Sham et al. 1994).

Decreased fecundity of patients with schizophrenia has been consistently reported (McGrath et al. 1999; Nimgaonkar 1998). Still, the prevalence of schizophrenia seems to be relatively stable over time. Recently, a decreased incidence of schizophrenia has been reported (Balestrieri et al. 1997; Brewin et al. 1997; Suvisaari et al. 1999). However, no consensus regarding decreasing incidence of schizophrenia has yet been reached and several more or less speculative explanations for this paradox have also been suggested. For example, the hypothesis of the "odyssean personality" was put forward in the 1970s (Jarvik and Deckard 1977). Other theories about the "heterozygote" or "subclinical" advantage of relatives of patients with schizophrenia have also been suggested (Andreasen 2000).

When studying the dates of births of schizophrenic patients, a 5-8% excess of winter-spring births has been observed for patients with schizophrenia compared to the general population (Torrey et al. 1997). The reason for this variation is unknown, but for example infectious agents have been suggested, specifically influenza A epidemics (Cannon et al. 1996; Takei et al. 1996)

To be able to collect representative study samples and classify individuals correctly with respect to disease status in a genetic study, one has to know which phenotypes share some part of a common genetic etiology. To this end several family studies have been performed investigating the familial relationship between schizophrenia and other, possibly related disorders. It has clearly been shown that the risk of several psychotic disorders is increased among the relatives of schizophrenic probands, specifically shizoaffective disorder, schizophreniform disorder, delusional disorder and atypical psychosis (e.g. Kendler et al. 1993b). Kraepelin (1919) and Bleuler (1950) already described non-psychotic family members of schizophrenic patients who were odd or eccentric. Using modern nosology, paranoid, schizoid, schizotypal and avoidant personality disorders have been shown to aggregate in families of schizophrenic probands (Kendler et al. 1993a; Lichtermann et al. 2000). This was the basis for the use of liability classes in our linkage studies (Studies I-III); schizophrenia was used as liability class 1 and the spectrum conditions were included as increasingly inclusive liability classes (2-3). We also treated the individuals diagnosed with depression or bipolar disorder as affected in a fourth liability class. This was done based on some reports of a familial relationship between affective disorders and schizophrenia (Maier et al. 1993), a finding not confirmed in other epidemiologic studies (Kendler et al. 1993b). The fact that schizophrenia has a familial relationship with the disorders mentioned above tells us that they share some form of familial predisposition. This familial predisposition might naturally be due to genetic factors increasing the risk to several of the disorders. However, there must also be unique genetic factors predisposing only to schizophrenia. Therefore, in studies I-III, we performed statistical analyses separately for the four increasingly inclusive liability classes.

1.3 Environmental risk factors

This thesis addresses the genetic risk factors of schizophrenia, but a brief discussion about the environmental risk factors of schizophrenia is warranted. As stated earlier, the etiology of schizophrenia is unknown, and so are the environmental risk factors increasing the risk of developing the disorder. However, some possible environmental risk factors have been identified and are supported by observational data, these being infections, obstetric complications, malnutrition and childhood rearing environment.

Especially second trimester exposure to influenza has been reported to be associated with later increased risk of developing schizophrenia. In a study in the Helsinki area, individuals whose mothers were exposed to the 1957 influenza epidemic during their second trimester of gestation had a significantly elevated risk of developing adult schizophrenia (Mednick et al. 1988). This finding has been replicated by several studies (Barr et al. 1990; Kunugi et al. 1995; McGrath et al. 1994; O'Callaghan et al. 1991; Takei et al. 1996; Takei et al. 1995).

There is compelling evidence of an association between pre- and perinatal complications and schizophrenia susceptibility (Hollister et al. 1996; Jones et al. 1998; Sacker et al. 1995). Specifically, low birth weight, short gestation, low Apgar scores and rhesus incompatibility have been shown to be susceptibility factors for schizophrenia.

Prenatal malnutrition has been shown to be associated with increased risk of developing schizophrenia. Specifically, children who were exposed to the famine in the Netherlands during 1944-45 in utero in their first trimester of gestation had a higher risk of developing schizophrenia (Susser et al. 1996). In a Finnish study, low maternal late-pregnancy BMI was shown to be an independent risk factor for schizophrenia (Wahlbeck et al. 2001).

Historically, many different psychosocial factors have been argued to be directly and causally linked to schizophrenia. This has been based on psychoanalytic and psychodynamic theories as well as on theories regarding the family. Very few well-designed studies have been published regarding these factors, and many of them seem outdated to the contemporary scientist. From a clinical point of view, these theories can be useful to understand how the disease can affect the patient and his/her social network. It is still possible that childhood rearing environment has an effect on the risk of developing schizophrenia. For example lower "general understanding and management" of their children by the mothers has been associated with a higher risk of developing schizophrenia (Jones et al. 1994). Also, being born as the result of an unwanted pregnancy has been associated with later schizophrenia in one study (Myhrman et al. 1996)

1.4 Neuropathology

The limbic system has been well studied in schizophrenia because of its central role in the control of emotions. Many studies of post-mortem brains from schizophrenic patients have reported decreased size of, among others, the amygdala, hippocampus and parahippocampal gyrus (Aso et al. 2001; Gur et al. 2000; Sigmundsson et al. 2001; Wright et al. 2000). For a review of these findings, see Shenton et al. (2001).

The basal ganglia have also been extensively studied in schizophrenia. These structures have a role in the control of movements, and are implicated in schizophrenia because of the odd or stereotypical movements that many schizophrenic patients express even without the effect of medication. Some movement disorders involving the basal ganglia, for example Huntington's disorder, are also quite often associated with psychosis. The results of studies of the basal ganglia have been less conclusive, with reports of decreased size of the substantia nigra (Bogerts et al. 1983), increased size of the globus pallidus (Elkashef et al. 1994), but also several negative studies (see for example: Gur et al. 1998; Lang et al. 2001).

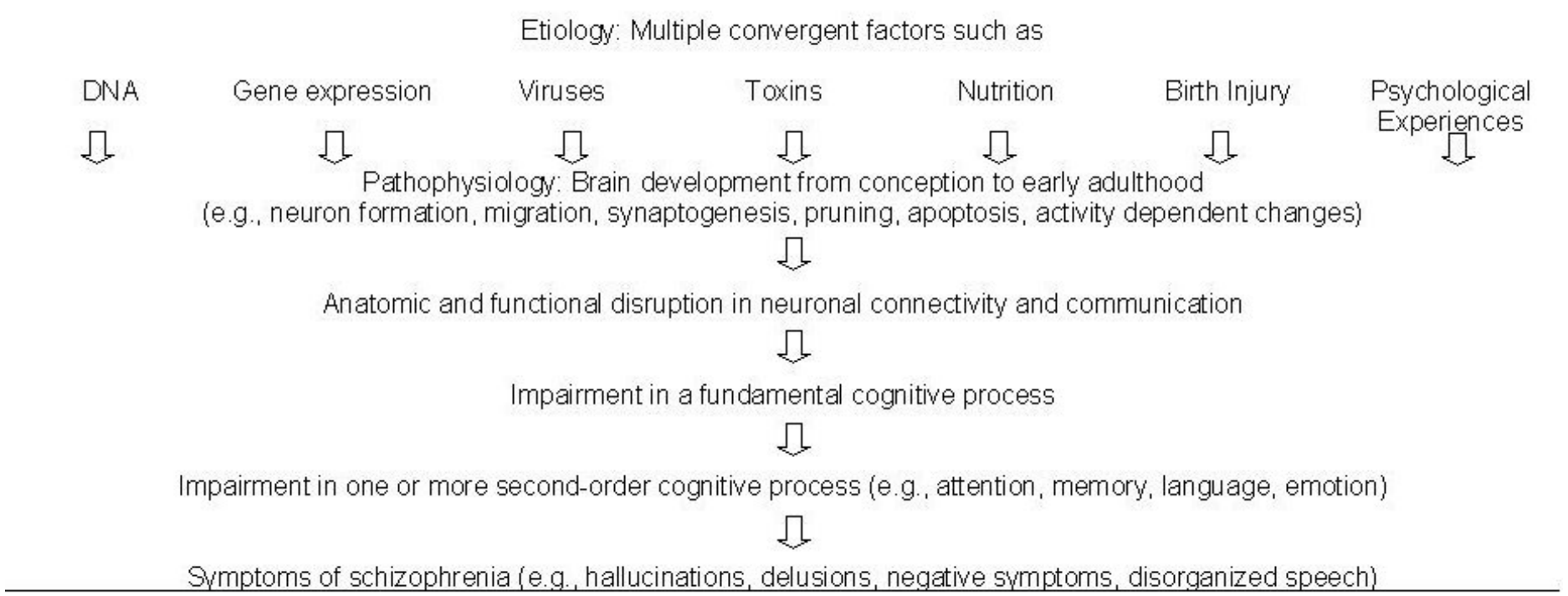
Total brain volume has also been found to be decreased in schizophrenic patients. Specifically, many consistent reports of decreased lateral ventricle volume have been published. Especially the cortical volume of brains of schizophrenic patients has consistently been shown to be decreased. For a review of these findings, see McCarley et al. (1999). Reports on the possible role of the cerebellum in higher cognitive functions and some MRI findings of increased vermis volume and cerebellar assymmetry in schizophrenic subjects have recently been published (Levitt et al. 1999).

1.5 Endophenotypes

Schizophrenia is defined by its clinical symptoms. The diagnostic systems developed for clinical purposes have aimed at optimizing reliability of the diagnosis to enable clinicians to communicate. This necessarily means that validity is somewhat sacrificed. The diagnostic systems focus on only a few of the symptoms that the patients present with, most prominently the positive psychotic symptoms as described above, since they are easy to operationalize. For genetic studies and other studies aiming at identifying pathophysiology and etiology, however, other definitions of the phenotype might be necessary.

Kraepelin and Bleuler already stressed the importance of defining the illness by attempting to identify a "morbid process" (Figure 1) (Andreasen 2000). Psychotic symptoms are not specific to schizophrenia. For both Kraepelin and Bleuler, the most characteristic feature of schizophrenic subjects was

Figure 1. The "working model" of schizophrenia as suggested by Andreasen (Andreasen 2000)



fragmenting of the cognitive processes, what we today would refer to as formal thought disorder.

With this in mind, it is clear that scientists in the field of schizophrenia genetics are interested in finding phenotypes that define some sub-group of the large population of schizophrenic subjects, while at the same time offering a closer relationship to the underlying pathophysiologic process. Such phenotypes have mostly been derived from neuropsychology and neurophysiology. An ideal endophenotypic measure should show significant and consistent abnormality in at least a sub-group of schizophrenic subjects compared to normal controls, and also in their first-degree relatives.

The so-called P50 evoked potential is a characteristic spike in the EEG of both normal and schizophrenic patients occurring 50μs after a short auditory stimulus. In normal individuals, this spike gradually vanishes after repeated exposure to the same stimulus. In schizophrenic patients, however, this suppression does not occur, and the same spike is seen regardless of the number of times the stimulus is repeated. The same deficit of suppression is seen also among the unaffected first-degree relatives of schizophrenic patients, and again among individuals with other schizophrenia spectrum disorders (Freedman et al. 2000). The importance of this measure has been further emphasized by the finding of a linkage between the alpha 7-nicotinic receptor and p50 deficit in schizophrenic patients (Freedman et al. 1997)

Between 50% and 80% of schizophrenic patients have eye-tracking deficits, compared to 8% of the general population (Levy et al. 1993). This means that they have an inability to accurately follow a moving visual target. This is caused by a disinhibition of saccadic eye movements and can therefore be thought of as yet another result of deficient inhibition. This deficit is also seen among 25-40% of first-degree relatives of schizophrenic patients (Holzman 2000). Eye-tracking deficit can thus be viewed as a pleiotropic manifestation of schizophrenia and might be useful as an endophenotype for genetic studies.

Working memory, the more contemporary term for short-term memory, is conceptualized as an active system for temporarily storing and manipulating information needed in the execution of complex cognitive tasks, e.g. learning, reasoning, and comprehension (Baddeley et al. 1986). As measured by the Digit Span subtest of Weschler Memory Scale - Revised (Russell 1975),for example, schizophrenic patients tend to show significantly impaired working memory compared to controls (Cannon et al. 2000), and their unaffected relatives are intermediate here, too (Krabbendam et al. 2001).

In 1996 Cloninger et al. suggested that "When a disease is caused by interactions among multiple susceptibility dimensions, each of which may be oligogenic, then the replication of particular genes is unlikely in samples of

practical size. Consequently, it may be more fruitful to map genes contributing to temperament, which has a relatively simple genetic architecture and can be quantified easily and reliably by questionnaire." (Cloninger et al. 1996). One such questionnaire, the TCI (Cloninger 1987) was designed and validated for the quantitative assessment of personality disorders (Battaglia et al. 1996). The temperament dimensions measured by the TCI have not been validated directly as endophenotypes for schizophrenia. However, it is conceivable that alleles contributing to the susceptibility for certain personality disorders (schizoid, schizotypal, paranoid) belonging to the so-called schizophrenia spectrum can have some role also in the etiology of e.g. schizophrenia. Therefore these kind of quantitative measures of personality have been suggested as a possible means to circumvent some of the problems associated with mapping of susceptibility genes for major mental disorders.

1.6 Comorbidity

The positive or negative correlation of two disorders can give some clues about the etiology of either or both of the disorders. Factors affecting the susceptibility of both disorders can be easier to identify if a correlation between them is observed. For example, the comorbidity between lung and renal cell carcinoma (RCC) has led to the identification of one allele of the N-acetyltransferase 2 (NAT2) gene as a genetic risk factor for RCC among smokers (Semenza et al. 2001). This gene codes for a polymorphic enzyme involved in tobacco-carcinogen metabolism and was therefore a logical candidate gene based on the observed comorbidity. In psychiatry, the increased morbid risk of for example schizoaffective disorder and schizoid personality disorder among relatives of patients with schizophrenia has led to the conceptualization of the "schizophrenia spectrum" conditions, probably sharing some genetic risk factors (Kendler et al. 1993a). Numerous reports have suggested that the rates of physical illness differ between patients with schizophrenia and the general population. While most of the evidence must be regarded as anecdotal (Baldwin 1979), some observations deserve further study, among them a decreased incidence of lung cancer (Harris 1988; Tsuang et al. 1983), a highly counterintuitive finding given the increased rate and intensity of smoking among individuals with schizophrenia (Harris 1988). In genetic terms, the hypothesis is quite attractive that some schizophrenia vulnerability gene would at the same time decrease the risk of lung cancer despite heavy exposure to the strongest known environmental risk factor, tobacco smoke. A well-designed, population-based, three-center cohort study found a decreased risk of lung cancer in patients with schizophrenia (Gulbinat et al. 1992). A lower than expected incidence of cancers of the prostate, cervix and corpus uteri as well as an increased incidence of female breast cancer was observed, but not consistently so across the study centers (Gulbinat et al. 1992). These findings might turn out to be valuable when

mutations or DNA variants are identified in positional candidates of schizophrenia genes, as in the example above.

2 Strategies for searching for genes behind complex disorders

2.1 Genome-wide approach

Genome-wide scanning refers to genotyping of hundreds of polymorphic markers ordered and evenly spaced throughout the chromosomal DNA strands. Traditionally, around 400 microsatellite markers have been used for genome-wide scanning of both monogenic and complex disorders (Kruglyak 1997). Co-segregation of certain alleles of a marker and disease phenotype is then evaluated in linkage analysis. This is the method of choice for identifying novel loci without any pre-set hypothesis about the etiology of a disorder. The disadvantage of the method is the lack of information needed to define parameters for linkage analysis like inheritance pattern, penetrance, number of phenocopies etc. The relatively low statistical power, especially for complex disorders in which the locus-specific λ_s is not expected to be >3, probably even less (Risch 1990a), makes it necessary to collect very large study samples to be able to detect true linkages. The method has been highly successful in mapping genes for monogenic disorders, but for genetically complex human disorders very few genes have been identified based on linkage findings (e.g. Horikawa et al. 2000).

Lately, the novel techniques for monitoring SNP markers have drawn much attention. These techniques, like DNA-microarray technology, offer a way to monitor several thousands of markers in large study samples, but it is not yet clear whether they will offer a practically feasible means of tackling complex traits. Moreover, the statistical analysis of the large amounts of data provided by these techniques is still under debate (Weiss and Terwilliger 2000). It seems clear that most genetic variation in human populations is due to variation in single nucleotide polymorphisms (SNPs) (Altshuler et al. 2000). It has also been verified that SNPs have a clearly lower mutation frequency than microsatellite markers, making them more suitable for association analysis where co-segregation of a marker allele and disease is monitored for many generations (Kruglyak 1997).

2.2 Candidate gene approach

An hypothesis-driven and potentially powerful approach to the identification of susceptibility genes for any trait is to test for association between the trait and genes that are known or thought to be involved in the regulation of this trait. This is certainly the case for very complex disorders, like schizophrenia,

where detection of linkage in family materials can be difficult or even impossible (Terwilliger 2001). Because of the lack of knowledge about the etiology of schizophrenia the number of possible candidate genes is restricted only by the imagination of the scientist. This places a very stringent burden of statistical proof on positive results, because of low prior probability and issues of multiple testing (Owen et al. 1997). Perhaps the most obvious candidate genes are the neurotransmitters known or suspected to be involved in the pathophysiology, if not necessarily the etiology, of schizophrenia. These have also been extensively studied by association methods, e.g. dopamine receptors, serotonin 5HT2A receptor, Kca3, to mention but a few. The list of candidate genes studied in schizophrenia is very long and cannot be discussed here. Some of them are listed in Table 4. For a detailed review of some of these studies see O'Donovan and Owen (1999).

Table 4. Some candidate genes for schizophrenia

Gene	*Symbol*
Dopamine receptor 1	DRD1
Dopamine receptor 2	DRD2
Dopamine receptor 3	DRD3
Dopamine receptor 4	DRD4
Dopamine receptor 5	DRD5
Dopamine transporter	SLC6A3
5-hydroxytryptamine type 2a receptor	5-HT2a
Tyrosine hydroxylase	TH
Prohibilinogen deaminase	PBGD
Calcium activated potassium channel	hKCa3/KCCN3
Disrupted in Schizophrenia 1 and 2	DISC1 and 2
Reelin	RELN
Neuronal nicotinic cholinergic receptor alpha2	CHRNA2
Homologous to Drosophila Notch - 4	NOTCH4
Brain-derived neurotrophic factor	BDNF
Neurotrophin-3	NT-3
Serotonin transporter promoter region	5-HTTLPR
Myo-inositol monophosphatase 2	IMPA2
Tumor necrosis factor alpha	TNFα
Catechol-O-methyltransferase	COMT
Spinocerebellar ataxia 1	SCA1

2.3 Statistical methods

The ultimate goal of any genetic study of complex disorders is to find a functional variant that has an effect on disease susceptibility. Any base within the genome can theoretically be a causative variant, but with present techniques it is not practicable to directly study every base in the genome.

Therefore alternative approaches have to be employed to localize the regions most likely to contain susceptibility genes. The most widely used methods are linkage analysis, association analysis, allele-sharing methods and animal models (Lander and Schork 1994).

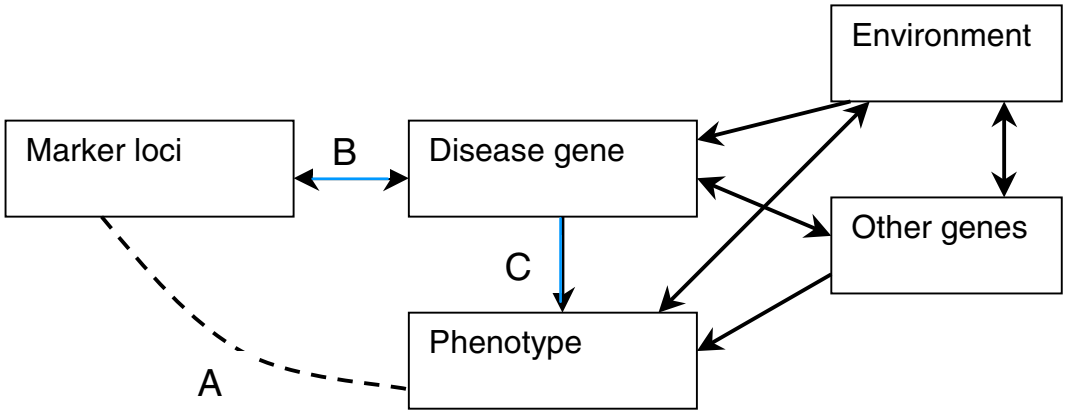
2.3.1 Linkage and association analysis

Traditional linkage analysis is often referred to as "parametric" analysis since one has to specify the parameters a priori. These are the allele frequency, penetrance and phenocopy rate. One monitors the co-segregation of marker alleles with the studied phenotype. The statistic most commonly used is the lod score (Z), which is given by the formula:

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

in which L is the likelihood function and Θ is the recombination fraction (Morton 1955). The most likely distance between two loci (e.g. a marker and a gene) is the recombination fraction at which the lod score peaks. However, since any error in the specified parameters, i.e. the disease model, will influence the estimation of the recombination fraction (Goring and Terwilliger 2000a), this property of the lod score is of no use in the localization of susceptibility genes for complex disorders (Figure 2). Classically, an odds ratio of more than 1000:1 in favor of linkage (equal to a lod score of 3) has been interpreted as statistically significant evidence for linkage in monogenic disorders (Conneally et al. 1985; Morton 1955).

Figure 2. Diagram showing a theoretical model for the relationship between marker loci, disease genes and phenotype. In linkage and association analysis we study relationship A, whereas the true relationship between marker loci and phenotype is B + C. Relationship C is complicated by environment and other genes as well as incomplete penetrance and phenocopies.



For complex disorders, somewhat modified criteria have to be used. Lander and Kruglyak have suggested three different categories for interpreting significance of genome-wide scans, namely suggestive, significant and highly significant linkage. These would be expected to occur one, 0.05 and 0.0001 times in a genome-wide scan respectively, and would correspond to lod scores of 2.2, 3.6 and 5.4 in a sib-pair study (Lander and Kruglyak 1995). These guidelines have been widely accepted by the field, but are arbitrary in nature, and have also been criticized (Elston 1997; Morton 1998). Only confirmation of the finding in a separate study sample will convince researchers in the field that the linkage is not a false positive finding.

Allele-sharing methods, such as the affected sib-pair method, are alternations of traditional linkage analysis and are often referred to as "model free", because no parameters have to be specified. However, it has been shown that for example the mean test, which can be considered the most prominent of the affected sib-pair tests, is equivalent to lod score analysis for an assumed recessive mode of inheritance, irrespective of the true mode of the disease (Knapp et al. 1994). Therefore, traditional linkage analysis and allele-sharing methods are actually conceptually very similar.

Association analysis on the other hand is equal to linkage analysis in a very large pedigree, namely the whole population from which the study sample is drawn (Terwilliger and Goring 2000). Because of the large number of meioses in such a pedigree, the region in which a signal can be detected is relatively small, but within that region the statistical power is greater than for traditional linkage analysis. One could therefore interpret association analysis as yet another variant of traditional linkage analysis. Since animal crossing studies in gene identification ultimately use association (or linkage) analyses for identification of disease alleles, all four methods described by Lander and Schork (1994) can actually be thought of as variations of one method.

Linkage analysis can be performed by comparing co-segregation of one marker with disease status (two-point linkage analysis), or information can be extracted from several markers at a time (multipoint linkage analysis) as implemented in several statistical software programs, e.g. Genehunter (Kruglyak et al. 1996), and SimWalk2 (Sobel and Lange 1996). Using complete information, two-point and multipoint analysis are identical, but in the case of missing data for one marker, multipoint methods can increase the information obtainable for loci close to that marker. However, they are sensitive to inconsistencies in recombination fractions between markers, commonly occurring genotyping errors and persisting errors in the marker map (Goring and Terwilliger 2000b).

2.3.2 Quantitative measures

Psychiatry has been dominated by different diagnostic classifications like the DSM (APA 1994) and the ICD (WHO 1994) diagnostic systems. In such diagnostic systems, a person is classified as affected if he/she fulfills certain diagnostic criteria. They are therefore by nature dichotomous. Systems like these are needed to construct treatment guidelines. However, DSM-IV states that "there is no assumption that each category of mental disorder is a completely discrete entity with absolute boundaries dividing it from other mental disorders or from no mental disorder" (p. xxii). Indeed, in natural populations, variation in most characters takes the form of a continuous phenotypic range rather than discrete phenotypes. Probably most psychiatric disorders are also extremes either of normal behavior or of some underlying susceptibility to the disorder. As described above (1.5 Endophenotypes) several quantitative measures that reflect schizophrenia liability have been identified. These include neuropsychologic traits like working memory, neurophysiologic traits like eye-tracking, and neuroradiologic traits like size and activation of different brain-regions, to mention a few. These are all measured quantitatively and can be assessed in both patients and their unaffected relatives.

There are several statistical computer programs available to perform genetic linkage analysis utilizing this kind of quantitative phenotypic data. Probably the most prominent is the SOLAR package (Almasy and Blangero 1998), which uses a variance components approach to calculate IBD matrices for pedigrees of any size, and then perform two-point or multipoint analysis for the marker data. The idea is that if the studied trait, or some underlying risk factor, is measurable on a quantitative scale it should be used as such. Dichotomizing the trait into affected and non-affected classes causes a lot of information to be lost and diminishes the power to detect linkage (Duggirala et al. 1997).

2.3.3 The issue of power in a linkage study

To be able to plan the research, to use the appropriate amount of resources, and to interpret the results, it is useful to have some grasp of the statistical power of a certain study sample in linkage analysis. For a monogenic disorder the estimation of statistical power of a study sample of a certain size is relatively trivial. Several software programs are available to calculate the probability of finding linkage above a certain threshold in a given study sample, e.g. Simlink (Boehnke 1986). This kind of statistical power analysis is possible also for oligo- and polygenic disorders by specifying several parameters a priori, these being the number of genes underlying the disorder, the disease gene frequency, and the heritability and penetrance of the disorder, in addition to information about the study sample. In a disorder like schizophrenia all these parameters are unlikely to be correctly estimated, with

the possible exception of heritability (e.g. (Cannon et al. 1998)). The number of genes underlying schizophrenia can be indirectly estimated from the steepness of decrease in incidence with decreasing biological relatedness with a proband. However, this is confounded by familial and unique environmental risk factors and also by epistatic interactions between susceptibility loci. The product of disease gene frequency and penetrance is correlated with the lifetime prevalence, but the magnitude of the two factors cannot be reliably determined in schizophrenia.

Despite this, a priori power calculations have been made for many schizophrenia studies, often showing that the studies would have had the power to detect linkage. The repeated failure to find genes for schizophrenia provides substantial evidence that the assumed architectures of the disease postulated by many researchers in the field are grossly oversimplified. For this reason, since all we know is that the etiology must be more complicated than has been expected previously, power calculations were considered a gratuitous exercise in our studies. For a detailed review of the problems of power estimation in linkage studies for complex disorders, see for example (Terwilliger and Goring 2000).

Based on the experience of previous genome-wide scans for schizophrenia, it was clear already from the start of this project that obtaining sufficient statistical power was going to be a major problem. We undertook several strategies to circumvent some of these problems; 1) We collected as large a study sample as was possible using the resources available. 2) The familiar enrichment of the disorder was maximized, especially in the internal isolate, by collecting families with as many affected individuals as possible. 3) We tried to maximize genetic homogeneity of the study sample by collecting large numbers of families from the more genetically isolated regions in the northeastern parts of Finland. Only the outcome of the present studies aiming at identifying disease-causing variants in the regions identified in the linkage studies will provide a definitive answer as to whether these strategies were successful.

2.4 Isolated populations

Genetically isolated populations offer many advantages for genome-wide mapping studies: the founder effect and high degree of inbreeding in small population isolates result in an increased incidence of some recessive disorders, causing some otherwise rare disorders to be found at relatively high rates. Typically, only in these special isolates can cases of rare diseases be found in sufficient number for the phenotype to be defined and for genetic mapping projects to be carried out.

Due to the founder effect and isolation, the monogenic diseases are less likely to show both locus and allelic heterogeneity. In fine mapping of positioned loci, strategies using LD and haplotype-sharing between affected individuals can thus be applied to efficiently restrict the critical DNA region (Peltonen et al. 1999; Peltonen 2000).

Based on the findings in monogenic diseases, an appropriate hypothesis considering the genetic background of multifactorial diseases would be that in isolated populations fewer disease-predisposing alleles and, potentially, fewer influencing genes behind a given trait will be encountered than in populations of more heterogeneous origin (Collins 1995; Lander and Botstein 1986; Lander and Schork 1994; Peltonen et al. 2000). Several genetic-mapping studies in multifactorial diseases have been carried out in study samples from isolated populations, including bipolar disease in Old Order Amish (Ginns et al. 1996) and Costa Rican populations (Freimer et al. 1996), for a review see (Peltonen et al. 2000). Finnish subpopulations have also been used in genetic studies of complex diseases like multiple sclerosis (Kuokkanen et al. 1997), diabetes (Mahtani et al. 1996), schizophrenia (Hovatta et al. 1999) and familial combined hyperlipidemia (Pajukanta et al. 1998). All these studies have been based on the collection of large pedigrees with multiple affected individuals, facilitating the use of traditional linkage analyses, and have in most cases resulted in the initial positioning of several susceptibility loci. So far, no specific susceptibility gene has been identified on the basis of these studies, and therefore the genetic background of multifactorial diseases in isolates like Finland cannot yet be characterized.

Importantly, no genome scan for predisposing loci in complex diseases among genetic isolates has provided evidence for LD, even when relatively high-density maps with marker intervals of 1-2 cM have been used. However, candidate gene-based studies have revealed association between intragenic markers and complex traits, for example in two Finnish studies on hypertension and multiple sclerosis (Kainulainen et al. 1999; Tienari et al. 1994b). In a genome scan for schizophrenia loci in families collected from one sub-isolate of Finland, no statistically significant association was detected using a marker map with a 1 to 2-cM marker interval. This isolate is only 15-17 generations old, but has shown an expansion rate of 170% during the past 100 years, and this has probably resulted in the rapid decay of LD in disease alleles (Hovatta et al. 1997). Based on this limited experience, it seems that genetic intervals revealing LD in common disease alleles in Finland are much more restricted than in the case of monogenic diseases. This is probably due to anticipated locus heterogeneity, and the fact that several predisposing mutations have been introduced even into genetic isolates. However, it should be emphasized that alleles from different regions of Finland reveal different interval of LD. Mounting evidence from the Finnish population suggests that LD does extend significantly further in certain sub-populations than in the general population of Finland (Mohlke et al. 2001; Varilo et al. 2000; Varilo

personal communication). This further underlines the importance of careful selection of study population and genealogical tracing of ancestors.

Recent theoretical studies of populations with different demographic histories have actually suggested that although rapidly expanding populations (such as the Finns) are ideally suited for mapping of rare monogenic disease genes, old and stable populations (such as the Lapps) may be better for mapping genes for complex diseases that are probably caused by older, common mutations (Laan and Paabo 1997; Slatkin 1994; Terwilliger et al. 1998). In isolated populations of small constant size, the drift probably generates new disequilibrium more quickly than recombinations and mutations result in its decay. The power of isolates with disparate genealogical history in the mapping and positional cloning of common disease genes remains to be demonstrated. Shared environmental and cultural homogeneity in many isolates might ultimately be even more beneficial than actual or assumed genetic homogeneity.

3 Genetics of schizophrenia

3.1 General aspects

Scientists believed for most of the past century that genes play a role in the etiology of schizophrenia (Tsuang et al. 1999). The first systematic family study dates back to 1916 and was performed by Rüdin and Kraepelin in Munich (reviewed in Gottesman 1991). Technical advances in the field of genetics, especially the use of polymorphic repeat sequences as genetic markers (Botstein et al. 1980),made it possible to begin studies of the genetic etiology of schizophrenia in the 1980s, mostly using single families densely loaded with schizophrenia. The first promising report of a genetic linkage to schizophrenia was published in 1988 (Sherrington et al. 1988), but this finding has not been replicated (McGuffin et al. 1990).

3.2 Heritability of schizophrenia

The majority of studies show that schizophrenia runs in families. The population lifetime prevalence of schizophrenia is about 1%. The risk for relatives of schizophrenic subjects to develop the disorder is higher the closer the genetic relationship is, i.e. the more genes the relative shares with the schizophrenic subject. The prevalence in relatives is shown in detail in Table 5 (Tsuang 2000).

Table 5. Morbid Risk of Schizophrenia for Relatives of Schizophrenic patients (Tsuang 2000).

Relationship	% Shared genes	Risk (%)
General population	N.A.	1
Spouses of patients	N.A.	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

However, the fact that the trait runs in families does not tell us whether this is due to genetic or to familial-environmental causes. To elucidate this matter a large number of well-designed family and twin studies have been performed. Some evidence from adoption studies also exists.

Twin studies are in many respects the best way to disentangle genetic effects from environmental ones. Higher concordance between monozygotic twins than between dizygotic twins clearly suggests a role of genes in the etiology of the disorder. Twin studies have consistently showed a high heritability of schizophrenia in several populations (Cannon et al. 1998; Cardno et al. 1999; Kendler and Diehl 1993). Specifically, the Finnish study by Cannon et al. (1998) estimated the heritability of schizophrenia at 83%, while the remaining 17% was attributable to unique environmental factors. Common environmental factors had no influence on liability. Similar findings have been observed in the other studies.

The Copenhagen High Risk Project (Cannon and Mednick 1993) found a prevalence of schizophrenia of 16.2% in offspring of schizophrenic mothers compared to 1.9% in the control group (Parnas et al. 1993). In the New York High Risk Study (Erlenmeyer-Kimling et al. 1997) schizophrenia was observed only among offspring of parents with schizophrenia, not in the control group. The same result was reported by the Israeli High Risk Study (Ingraham et al. 1995)

Two Danish adoption studies of schizophrenia (Kendler et al. 1994; Kety et al. 1994) found an increased risk of schizophrenia among biological relatives of probands with the same disorder, but no increase in adoptive relatives compared to the general population. The Finnish Adoptive Study of Schizophrenia (Tienari et al. 1994a) reported similar findings.

3.3 Previous findings of linkage in schizophrenia

The following is an overview of the linkage findings reported in schizophrenia. Linkage has been reported to most human chromosomes by some group, and replications have been rare, with some important exceptions. Table 6 shows the genome-wide scans for schizophrenia published to date.

Chromosome1: Brzustowicz et al. (2000) reported strong linkage (Zmax = 6.50) to chromosome **1q21-22** in a genomewide scan of 22 Canadian pedigrees. The families included in the study had been selected so that they all showed a dominant mode of inheritance. Still, quite paradoxically, the recessive model and narrow disease definition showed the strongest evidence for linkage. Gurling et al. (2001) reported evidence for linkage (Zmax = 3.2) to marker D1S196, situated close to the linkage peak identified in the study by Brzustowicz et al. Weak support for linkage to 1q22-23 was already reported in an earlier study (Shaw et al. 1998). The hKCa3/KCNN3 gene, a potassium-channel gene earlier reported to be associated with schizophrenia (Dror et al. 1999) is located in 1q21.

Somewhat further telomeric of the findings mentioned above, in the region **1q32-42**, there are several independent findings of linkage to schizophrenia. Hovatta et al. (1999) reported a three-stage genomewide scan in 69 families from a Finnish population isolate. They observed a maximum LOD score of 3.82 at marker D1S2891, under a dominant model and a narrow disease definition. A putative 6 cM haplotype, which might narrow the chromosomal region implicated, was observed in some core families. In their study of multiplex kindreds from Daghestan, Bulayeva et al. (2000 and personal communication) found a lod score of 1.8 for marker D1S2141 in the same region. In addition, one interesting linkage finding in bipolar disorder maps to the same marker (Detera-Wadleigh et al. 1999).

Chromosome 2: In their study of 17 extended pedigrees from Palau, Micronesia, Coon et al. found the strongest evidence for linkage on chromosome 2p for marker D2S441 (Zmax=2.17) (Coon et al. 1998). This finding from an isolated population was later replicated by Shaw et al. in a more mixed US population (Shaw et al. 2001) (D2S139, lod=2.25).

Chromosome 4: Hovatta et al. (1999) also observed a maximum LOD score of 2.74 at marker D4S1586 on chromosome **4q31**, under a dominant model and

Table 6. The genome-wide scans for schizophrenia published before November 2001. The two most significant regions from each scan are marked by an X. Regions appearing in two or more studies are marked in bold letters.

First author	Year	Ethnicity	# Families	# Affected	1q22-24	**1q32-42**	**2p12-14**	**2q12-13**	2q37	3p25	4p11-15	4q31	**5q31-33**	**6p22-24**	6q25	7q22	**8p21-22**	8q12	9q32-34	**10p12-14**	10q24	11p14	12q24	13q13	**13q32**	14q32	18q12	20q13	**Two major findings**
Coon	1994	US	9	36							X															X			lod= 1.73 (D4S35), 1.25 (D14S17)
Moises	1995	Mixed	65	213										X														X	p-value = 0.005 (D6S274), 0.0008 (D20S40)
Kaufmann	1998	US, Afric.amer.	30	79														X	X										npl= 2.3 (8pter-q12), 2.2 (9q32-q34)
Faraone	1998	US, cauc.	43	96				X												X									npl= 3.4 (D10S1423), 2.4 (D2S293)
Shaw	1998	US	72	161																		X		X					lod= 2.85 (D13S1293), 2.30 (D11S1393)
Levinson	1998	US & australia	43	126				X													X								npl= 2 (D2S410), 2 (D10S1239)
Blouin	1998	US	105	275													X								X				npl= 4.18(13q32), 3.64(8p21-22)
Coon	1998	Palau	17	79			X																						lod= 2.17(D2S441)
Straub	1998	Ireland	265	542									X	X															lod= 3.51(D6S296), 3.04 (D5S393)
Hovatta	1999	Finland	21	82		X						X																	lod= 3.82(1q32.2-q41), 2.74(4q31)
Williams	1999	England	138	327							X																X		MLS= 1.73(D4S3009), 1.62(D18S451)
Ekelund	2000	Finland	134	268		X										X													lod= 3.18(D7S486), 2.62(D1S439)
Brzustowicz	2000	US	22	80	X																				X				lod= 6.05(D1S1679), 3.81(D13S793)
Schwab	2000	Germany	71	~157										X						X									lod=2.2 (D6S274), 2.1 (D10S1714)
Bailer	2000	Austria	5	16										X									X						p-value = 0.005 (D6S309), 0.02 (D12S366)
deLisi	2001	US	291	~580			X													X									lod= 2.25 (D10S189), 3.55 (D2S139)
Paunio	2001	Finland	238	591					X				X																lod= 4.43 (D2S427), 3.56 (D5S414)
Gurling	2001	UK & Iceland	13	68									X				X												lod= 3.6 (5q33.2), 3.6 (8p22.1)
Lindholm	2001	Sweden	1	43						X					X														lod= 3.30 (D3S1263), 3.45 (D6S264)

a narrow diagnostic model. On the other arm of this chromosome, Williams et al. reported the strongest evidence for linkage in their genome-wide scan (MLS=1.73, D4S3009) (Williams et al. 1999).

Chromosome 5: Silverman et al. (1996) reported a maximum LOD score of 4.37 at locus D5S111 on chromosome **5p14**, under dominant inheritance and a broad disease definition, in one large Puerto Rican pedigree.

Based on a genome scan of 265 Irish pedigrees, Straub et al. (1997) reported a maximum heterogeneity LOD score of 3.35 at marker D5S804 on chromosome **5q31**, under a recessive genetic model and a narrow diagnostic model. Schwab et al. (1997) reported additional support for this region (marker D5S399). In a recent genome-wide scan of British and Icelandic pedigrees Gurling et al. (2001), reported a lod score of 3.6 in this same region.

Chromosome 6: Straub et al. (1995), tested for linkage to **6p22-24** in 265 Irish families. They reported a maximum LOD score of 3.51 at marker D6S296, under an additive genetic model and a broad definition of schizophrenia. Additional support for linkage to this region was reported by Schwab et al. (1995; 2000), by Moises et al. (1995), and by Antonarakis et al. (1995). Cao et al. (1997) reported weak evidence for linkage to 6q21-22 in two independent samples: $p = 0.00018$ at locus D6S474, and $p = 0.00095$ at D6S424. Martinez et al. reported a nonparametric lod score of 3.82 in 141 independent sib-pairs (Martinez et al. 1999).

On the other arm of chromosome 6, Lindholm et al. recently reported a lod score of 3.45 (D6S264) from a very large Swedish pedigree (Lindholm et al. 2001)

Chromosome 8: Blouin et al. (1998) reported a nonparametric lod score of 3.64 at D8S1771 on chromosome **8p21**. When a broad disease definition was used in this sample, support for linkage increased, yielding a nonparametric lod score of 6.17 (Pulver et al. 2000). In addition, Kendler et al. (1996) reported a lod score of 2.34, using a dominant genetic model and a broad disease definition, in 265 Irish pedigrees. Further support for linkage to 8p21 was reported by Brzustowicz et al. (1999), with a maximum LOD score of 3.49 at marker D8S136, under a dominant model and narrow disease phenotype, in 21 Canadian families. Also, in their recently published genome-wide scan, Gurling et al. reported a lod score of 3.6 in this region (Gurling et al. 2001).

Chromosome 9: Hovatta et al. (1999) also observed a maximum LOD score of 1.95 at marker D9S922 on chromosome **9q21**, under a dominant model in the families from a regional subisolate of Finland.

Chromosome 10: Faraone et al. (1998) reported nonparametric lod scores of 3.4 and 3.2 at markers D10S1423 and D10S582, respectively, both on chromosome **10p12**. Schwab et al. (1998) found support for this region with a

nonparametric lod score of 3.2 at marker D10S1714. Straub et al. (1998) observed a multipoint heterogeneity lod score of 1.91 with markers D10S1426 and D10S674. Recently, Shaw et al. reported the strongest evidence for linkage (lod=3.55) in their genome-wide scan in the same region (D10S189).

Chromosome 13: Blouin et al. (1998) reported a nonparametric lod score of 4.18 near D13S174 on **13q32**. Further support for this finding was published by Brzustowicz et al. (1999), with a maximum heterogeneity lod score of 4.42 at D13S793.

Chromosome 15: Stober et al. (2000) reported linkage (NPL = 3.57) to **15q15** (D15S1012) in a study of periodic catatonia, a severe sub-phenotype of schizophrenia. This region is located close to the 7 nicotinic acetylcholine receptor gene (CHRNA7), a potential candidate gene, also implied in a study of the p50 sensory gating deficit (Freedman et al. 1997).

Chromosome 22: Pulver et al. (1994) observed a maximum lod score of 2.82 at marker locus IL2RB on chromosome **22q13**. There have been other reports of weak linkage to the same region (Coon et al. 1994; Polymeropoulos et al. 1994; Stober et al. 2000). This region is close to the velocardiofacial syndrome (VCFS) deletion, which is reportedly associated with psychotic features (Murphy et al. 1999).

Chromosome X: The Finnish isolate study (Hovatta et al. 1999) reported a maximum lod score of 2.01 at the MAOB locus on chromosome **Xp11**. Some other studies have also found positive lod scores in the close vicinity of MAOB (Dann et al. 1997; DeLisi et al. 1994).

As is obvious from the overview above, some regions have been identified by several independent studies. Due to the large number of studies published to date and the possibly large number of negative replications not published, the significance of these replications is still hard to estimate. It is obvious that the linkage findings in schizophrenia are dispersed throughout the genome. There are several possible explanations for this. First, it could be a reflection of the truly genetically heterogenous etiology of the disorder. Most studies have been performed in different populations with different genetic background. However, methodological differences could also cause inconsistency in the results from different studies. For example, the definition of the phenotype has traditionally varied somewhat, even though in the more recent studies DSM-IV has generally been used as the gold standard. Whether or not diagnostic interviews have been performed can also affect the result of any given study. Since in most studies certain chromosomal regions are selected for further study based on the statistical analyses of the initial stages, even small differences in the statistical tools used can have an impact on the interpretation of the results. Such differences include for example the use of two-point versus multipoint or parametric versus non-parametric statistics.

Taken together, we cannot with certainty conclude whether these results reflect the true nature of the disorder studied or the shortcomings of the methods used to study it, but probably both factors play a role.

AIMS OF THE PRESENT STUDY

1. To use the genome-wide approach to search for genetic loci predisposing to schizophrenia in epidemiologic study samples collected in Finland.

2. To finemap one of the identified genomic regions and to investigate whether similar evidence for linkage or any evidence for association can be observed in disease alleles from different sub-populations within Finland.

3. To address comorbid traits of schizophrenia by testing association of a basic temperament measure and the DRD4 gene and by monitoring cancer risk in schizophrenic patients.

MATERIALS AND METHODS

Please refer to the original articles (I-V) and references for a more detailed description of the studies.

1 Study samples

1.1 Schizophrenia study sample

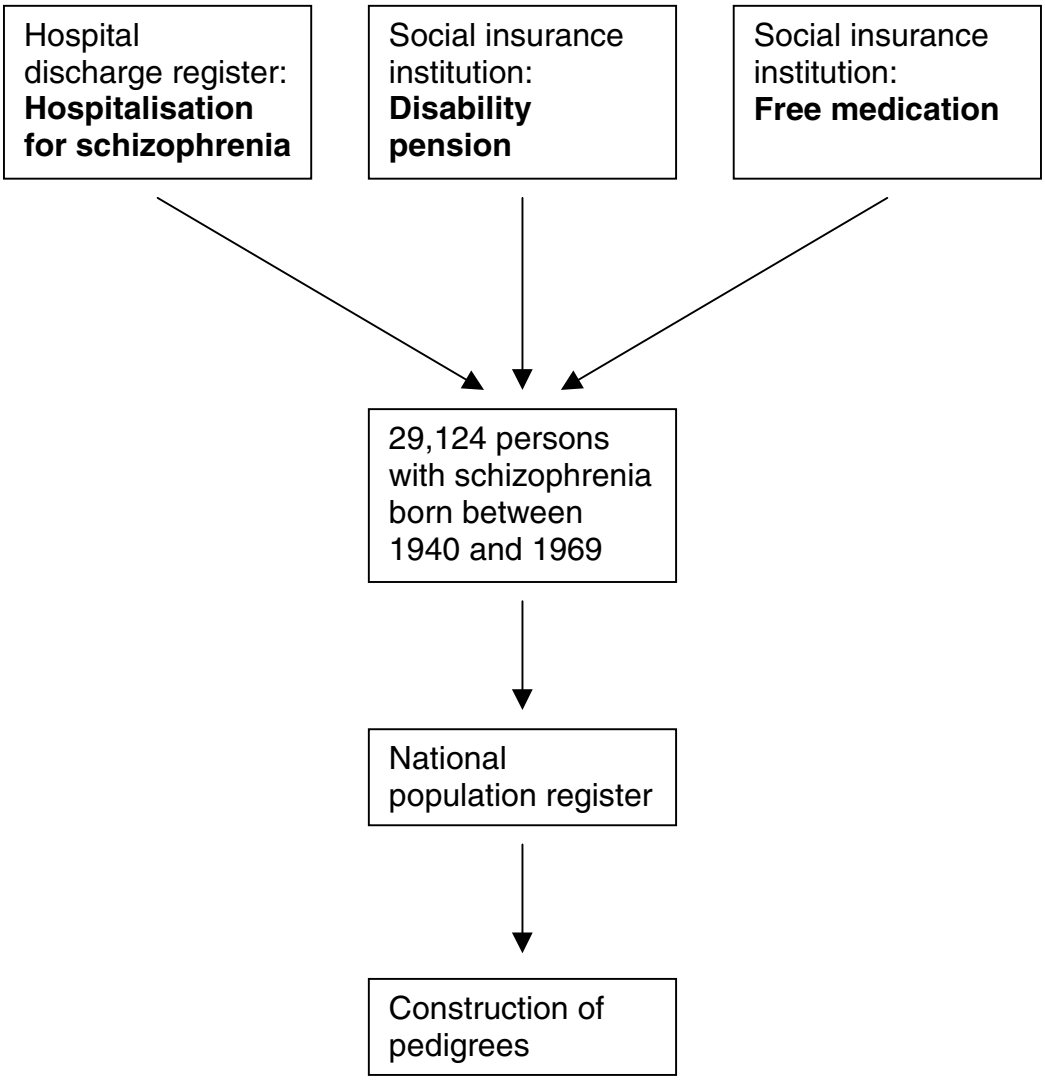
1.1.1 Identification of eligible individuals

The schizophrenia study sample was collected by utilizing three nationwide registers. All persons born between 1940 and 1969 and receiving free medication or a disability pension because of schizophrenia from the Social Insurance Institution of Finland were identified (N=17,930). Later, persons from the same cohort who had been hospitalized for schizophrenia at least once according to the Hospital Discharge Register were also identified, yielding a total of 29,124 individuals with a diagnosis of schizophrenia in any of the three registers. These data were linked to the National Population Register in order to identify all first-degree relatives of the patients and construct pedigrees (Figure 3).

1.1.2 Ascertainment of diagnosis

All available inpatient and outpatient records were collected for probands and relatives with any psychiatric diagnosis in any of the three registers. Two independent psychiatrists or psychiatric residents made DSM-IV best-estimate lifetime diagnosis. In case of disagreement, a third reviewer made a diagnosis to achieve a consensus diagnosis. One of the reviewers also filled out the OPCRIT checklist (McGuffin et al. 1991) for use in the future. The register diagnosis of schizophrenia has repeatedly been shown to have high reliability in Finland (Cannon et al. 1998; Isohanni et al. 1997; Mäkikyrö et al. 1998; Pakaslahti 1987). Diagnostic agreement between the different reviewers was good or excellent (see the attached articles for details on the different study samples).

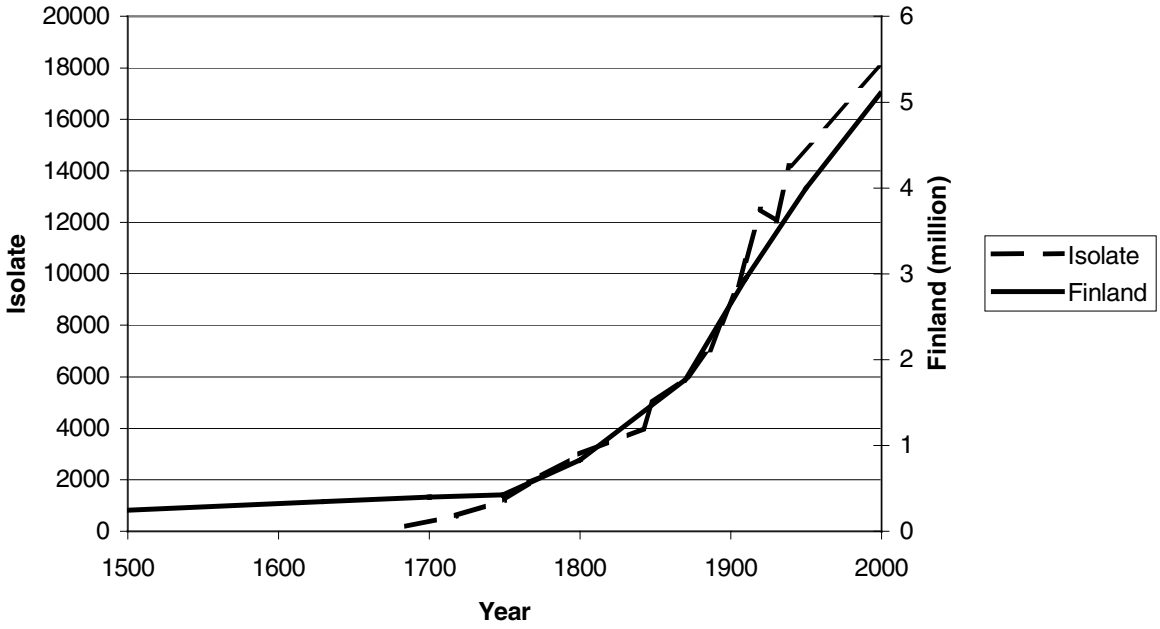
Figure 3. Flow-chart describing the identification of subjects for the schizophrenia study from the registers.



1.1.3 Internal isolate

Based on the settlement history of Finland, we treated the national study sample as two separate samples during the collection stage and also partly in the analytic stage, as described below. The population of the internal isolate in the north-eastern part of Finland was treated as a separate population based on genealogic data. The population of this area consisted of two Saami villages in the wintertime until the immigration of Finnish settlers at the end of the 17th century. The Finns came mainly from Ostrobotnia and South Kainuu, both groups having originated from Savo (Figure 5). Mr. Matti Hiltunen, the first Finnish pioneer to the district moved there in 1676. In 1685, the Finnish

Figure 4. Graph showing the growth of the Finnish population and the population in the internal isolate



population of the district consisted of 194 individuals in 34 families, and the Saami population in the district was approximately twice as large. The great famine in 1695-1697 killed about half of the Finns in the region and the majority of the Saami. In 1718, parish registers were established in the district, where there were currently 165 households and 615 individuals. Since then the population has expanded rapidly to its present size of over 18,000 (Figure 4). The population of this district remained almost completely isolated until World War II, and even since then, immigration to the district has been insignificant. These circumstances and history mean that the population of the district resembles an immense pedigree with several inbreeding loops. It is possible to obtain genealogic information on all living persons in the district back to 1718 using the registers administered by the Evangelic Lutheran Church of Finland and later the Finnish population register.

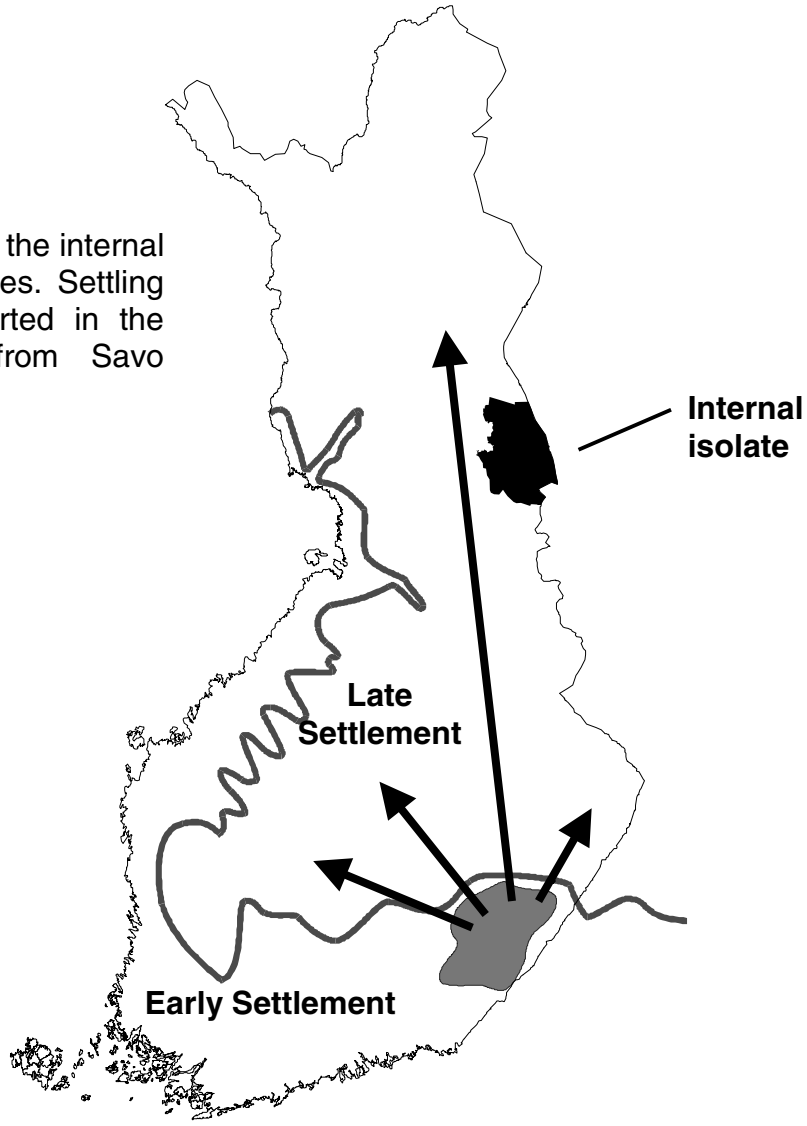


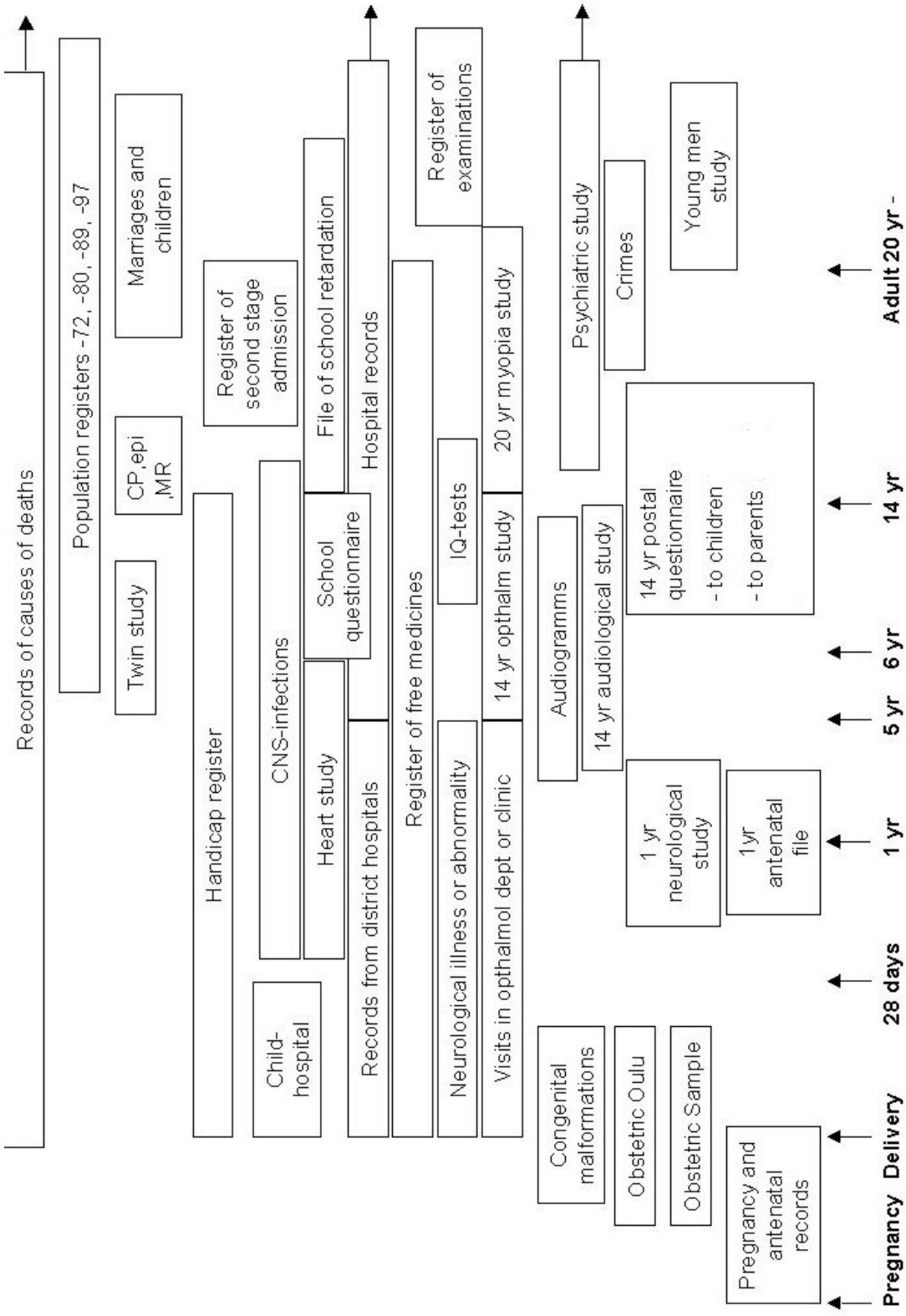
Figure 5. The settlement of the internal isolate utilized in our studies. Settling of the internal isolate started in the 1680s, and originated from Savo (shaded area).

1.2 Oulu 66 Cohort

Cohort-66 is a longitudinal one-year-birth-cohort study from an unselected population. The original purpose of the study was to describe and analyse the risk factors for perinatal deaths and low birth weight. The original names of the study were "North Finland premature-birth study" and "Development study of children in North-Finland". The project was later named "The mother-child cohort study of morbidity and mortality during childhood with the special purpose of preventing mental and physical handicap" and "Cohort-66 study".

Cases belonging to the survey were determined by the calculated term: the series comprised all mothers in Finland's two northernmost provinces of Oulu and Lapland with calculated term falling between January 1 - December 31, 1966. A small percentage of the births in fact occurred towards the end of 1965 and early in 1967. The calculated term, as was customary at that time, was counted from the first day of the last menstrual period. Where this date

Figure 6. Overview of the data collection process of the Oulu66 Cohort study in the previous stages of the study. The TCI data for Study V was collected at age 31.



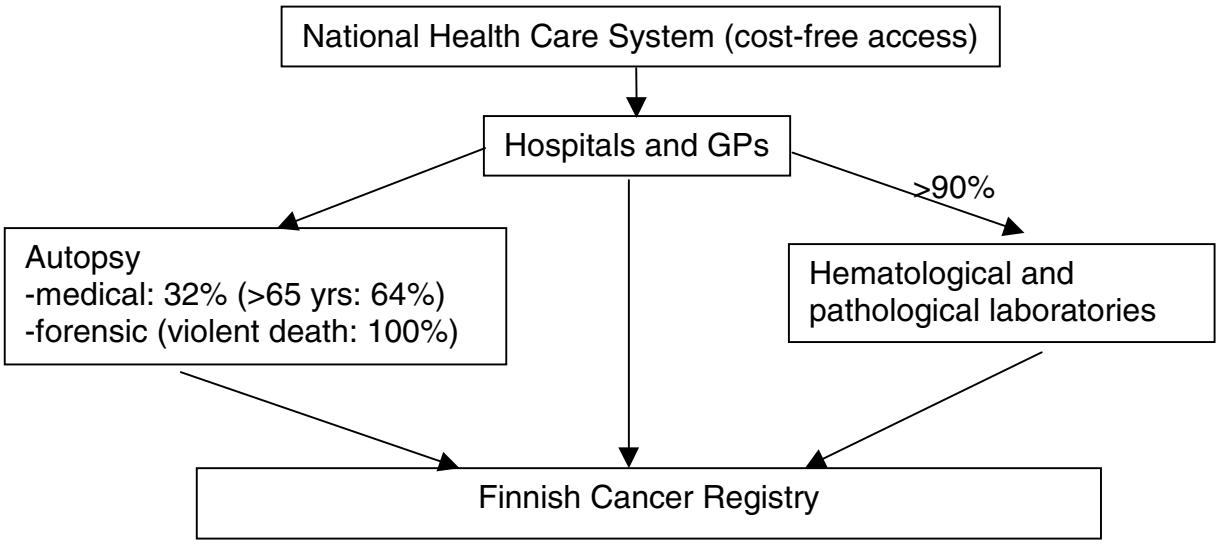
was unknown the expected term was estimated from the date of commencement of foetal movements and progress of the pregnancy. The study covered all live born and stillborn infants with a birth weight of 600 grams or more (Rantakallio 1969, 1988).

This sample is ideally suited to our purposes since the original cohort represents, without selection, all individuals born during one year in a geographically defined and culturally relatively homogeneous area. A large number of variables have been collected during regular follow-up examinations of participants throughout various stages of life from the prenatal period up to age 31 (Figure 6). In 1997, 8,411 of the original cohort members (N=11,637 alive in 1997) were living in the two northernmost provinces of Finland or in the capital area and were therefore considered eligible for the 31-year follow-up study in 1997-8. Among those who followed the invitation to a clinical examination, 5,041 subjects (83%) completed a personality questionnaire and gave written informed consent. This represents 43% of the cohort members alive at age 31, and 60% of the eligible. After exclusion of individuals with incomplete questionnaire and/or other background information, 4,691 individuals remained for our analyses.

1.3 Cancer comorbidity sample

The Finnish Cancer Registry was founded in 1952. It collects data on all cancer cases in the population of Finland and publishes statistics on cancer incidence. The Registry is countrywide and population-based (population 5.1 mill.). Informants who submit data on cancer patients to the cancer registry include all hospitals, physicians, pathological, cytological and hematological laboratories, and dentists. Data are also obtained via death certificates. The annual statistics on the occurrence of cancer give numbers of cases and incidence rates by gender, primary site, age and health care district. Tabular material by other or more detailed parameters can also be produced. This service is usually charge-free. Data identifying individual cancer patients can only be obtained for research purposes; permission granted by STAKES (National Research and Development Centre for Welfare and Health) is always needed.

Figure 7. Schematic overview of the registration of cancer cases in the Finnish Cancer Registry.



All medical care in Finland is provided by a national health care system to which everybody has almost cost-free access. This results in extraordinarily complete, unbiased ascertainment of cancer cases by the registers. The Finnish cancer registry has since 1961 continuously received obligatory reports on all newly diagnosed cases of cancer from hospitals, practicing physicians and hematological and pathological laboratories all over Finland, with about 90% of diagnoses based upon histological confirmation (Research 1997). Death certificates mentioning cancer are automatically forwarded from Statistics Finland to the Cancer Registry for review, although less than 2% of all cancer cases come to their attention in this way alone (Figure 7). Ascertainment of cancer cases has been shown to be virtually complete (Teppo et al. 1996).

In Finland medical or forensic autopsy is performed in 32% of all deaths, and in those occurring before the age of 65 years the proportion is 64% (Statistics Finland 1999). Forensic autopsy is also performed on all cases of violent death, including suicide. Since about 40% of the excess mortality in schizophrenia is accounted for by unnatural causes (Brown 1997) the autopsy rate is, if anything, higher among schizophrenic patients than in the control population, but the difference is expected to be small since the rate is also exceptionally high in the control group.

2 Laboratory methods

2.1 Sample collection and DNA extraction

20-30 ml of blood was drawn by venopuncture into EDTA tubes. DNA was extracted according to a standard procedure (Blin and Stafford 1976).

2.2 Genotyping

In study I, gel electrophoresis in stage I of the genome scan was performed on an ALF express automated DNA sequencer (Pharmacia Biotech) and genotyping achieved by assigning allele numbers according to the fluorescence curves generated. In stages II through IV gel electrophoresis was done on an ABI 377 automated DNA sequencer (Applied Biosystems), and genotypes were assigned using the Genotyper 2.0 software (Applied Biosystems). Markers were from Weber screening set 6 (Sheffield et al. 1995). Markers that failed to work satisfactorily were replaced by markers from the Généthon marker map. A total of 370 polymorphic microsatellite markers on the autosomes and chromosome X (average spacing 10.5 cM) were analyzed in stage I.

In studies II and III, PCR was performed according to standard procedures and electrophoresis was done on an ABI 377 sequencer (Applied Biosystems). Marker sequences were obtained from The Genome Database <www.gdb.org>, and marker order and inter-marker distances were obtained by RH mapping and by utilizing the Human Genome Project sequence data. When sequence data were used, the genetic distance was estimated from the physical distance assuming the equivalence of 1 million base pairs to 1 cM.

In study V, PCR was performed as described by Lichter et al. (1993) with slight modifications. One primer was fluorescently labeled with Cy5 for size separation on ALF express (Pharmacia Biotech). Genotyping was done using the Allelinks (Pharmacia Biotech) software.

3 Statistical methods

In studies I-III, mendelian inheritance of alleles in the pedigrees was confirmed by the PedCheck program (O'Connell and Weeks 1998). In all statistical analyses we classified subjects as either affected or unknown. We did not classify anyone as unaffected because unaffected family members

were not systematically assessed. Therefore all the results are based on 'affected only' analyses.

In both two-point and multipoint analyses, we wanted to minimize the problem of type 1 errors due to multiple testing. To this end, we used oversimplified inheritance models in all analyses. In two-point analyses one 'recessive' and one 'dominant' model were analyzed, as detailed below. Correspondingly, in multipoint analyses we considered only SimWalk's statistics A and B, the two most powerful statistics for detecting linkage to a recessive trait and a dominant trait, respectively. Several liability classes were adapted for all inheritance models and both the pedigree and nuclear family structures were used in the analyses. The analyses of several different liability classes for one inheritance model are not independent tests, but the obtained results still have to be interpreted with due caution because of the multiple tests performed.

It has been demonstrated that the sib-pair mean test is statistically equivalent to linkage analysis under a recessive mode of inheritance with no phenocopies allowed, and an infinitesimally rare disease allele (Knapp et al. 1994). As implemented in the SIBPAIR program (Kuokkanen et al. 1996), this has been shown to be one of the more reliable approaches to affected sib-pair analysis, especially when extended to sibships with two or more affecteds (Davis and Weeks 1997). This 'recessive' model was therefore used in the two-point analysis of the data. However, it is also possible to perform a corresponding analysis assuming that the sib-pair shares alleles identical by descent (IBD) from one of the parents, but not the other. Such a model has been shown to be equivalent to linkage analysis with a dominant model, rare disease allele, and no phenocopies (Kainulainen et al. 1999; Trembath et al. 1997). Therefore, such a 'dominant' affected relative pair analysis was performed using the MLINK program of the LINKAGE package, with technical details described elsewhere (Kainulainen et al. 1999; Trembath et al. 1997).

For association analyses we used a generalization of the TDT test, the gamete competition model (Sinsheimer et al. 2000), which can be employed to test for biased transmission of marker alleles to affected individuals. Because the null hypothesis is no association and no linkage, the method is not purely a test of association as linkage in itself also affects the observed p-value.

Due to of the great complexity of some of our pedigrees, most current software packages for multipoint analysis cannot use the full pedigree structure, but must rather simplify it in some way. One available program that can utilize all of the information available from such complex pedigrees is SimWalk2 (Sobel and Lange 1996), which uses a likelihood-based approach for sampling from all possible configurations on general pedigrees. We considered statistics 'A' and 'B' of SimWalk2. The statistics measure the degree of clustering among the affecteds of the founder-alleles, i.e. the

marker alleles descending from the founders. Specifically, 'statistic A' is based on the number of different founder-alleles contributing alleles to the affecteds, while 'statistic B' is based on the maximum number of alleles among the affecteds descended from any one founder-allele. Since SimWalk2 uses all available pedigree information, the calculation time for the complex pedigrees in our study sample is of the order of months when analyzing all available markers simultaneously (using a Compaq Alpha Server DS-10). We therefore selected smaller subsets of the genotyped markers to be analyzed by this method, based on the results of the two-point analyses. In all analyses, we used the non-parametric analysis alternative of SimWalk2. The number of replicates was 10,000 in all analyses.

In study IV, individual follow-up of patients, siblings and parents for cancer started on January 1, 1971. For patients with a first diagnosis of schizophrenia (or spectrum disorders) in the Hospital Discharge Register or Disability Pension Register after this date, follow-up began from the date of first diagnosis. For all three groups, individual follow-up ceased on December 31, 1996, or on the date of emigration or death if these occurred earlier. Person-years were calculated by duration of follow-up (<2, 2-11, >11 completed years of follow-up) and by 5-year age strata. Expected numbers of cancer cases (total and specific cancers as classified by primary site) in each stratum were obtained by multiplying the number of person years at risk by the corresponding average incidence rate of cancer in the general population of Finland during the same observation period. SIRs were calculated by dividing the number of observed cancer cases in each group by the expected number of cases. Calculation of 95% confidence intervals (CI) of SIR was based on the assumption that the numbers of observed cases followed a Poisson distribution.

For study V, we used the Pearson Chi-Square statistic for a table containing the high and low scoring NS groups and the possible alleles of the studied marker. The analysis was done using the SPSS 9.0.1 program (SPSS Inc., Chicago, Il)

ETHICAL CONSIDERATIONS

In addition to the general principles regulating biomedical research on human subjects, a disorder like schizophrenia poses the scientist certain additional ethical issues. Schizophrenia manifests itself in signs and symptoms that encompass the entire range of human mental activity; it damages a variety of functions that we regard as specifically human. The disorder is thus highly stigmatizing to the subjects, and the importance of confidentiality cannot be stressed enough. In our studies, we have followed the principles recommended in the 1964 World Medical Association Declaration of Helsinki, and its amendments. According to these principles, the subject can only be contacted by the treating physician most familiar to the subject. The relatives of the proband can only be contacted if and when the proband gives informed consent.

The capacity of schizophrenic subjects to provide informed consent has been discussed in the research community (Carpenter et al. 2000; Roberts 2000). A current psychotic episode clearly makes informed consent difficult to obtain. However, cognitive impairment can also make it difficult for some subjects to understand the information provided prior to the request for consent. In recognition of these concerns, we provided both written and oral information to the subjects in our study. In general, the Helsinki declaration is appropriate for protecting the rights of subjects, as the treating physician can always refuse to contact the subject if the clinical condition so requires.

The decision to perform a study should take into account the ultimate goal of the research and any possible abuse of the results, i.e. the ratio of possible gain to the subjects and the possible harm which might be caused to the subjects based on the obtained results. The potential gain from schizophrenia research is naturally better understanding of the etiology of the disorder and ultimately better treatment and/or prevention. However, a historical perspective also makes it easy to imagine undesirable consequences of genetic research on mental disorders, for example further stigmatization based on biological markers, discrimination, and even eugenics. One cannot be obtained without risk of the other, but in our opinion the potential benefits of schizophrenia research outweigh these risks. Greater understanding of the causes of schizophrenia would hopefully even reduce stigmatization and discrimination.

The research was approved by the Ministry of Social Affairs and Health and by the institutional review boards of the National Public Health Institute and the University of Oulu (Study I).

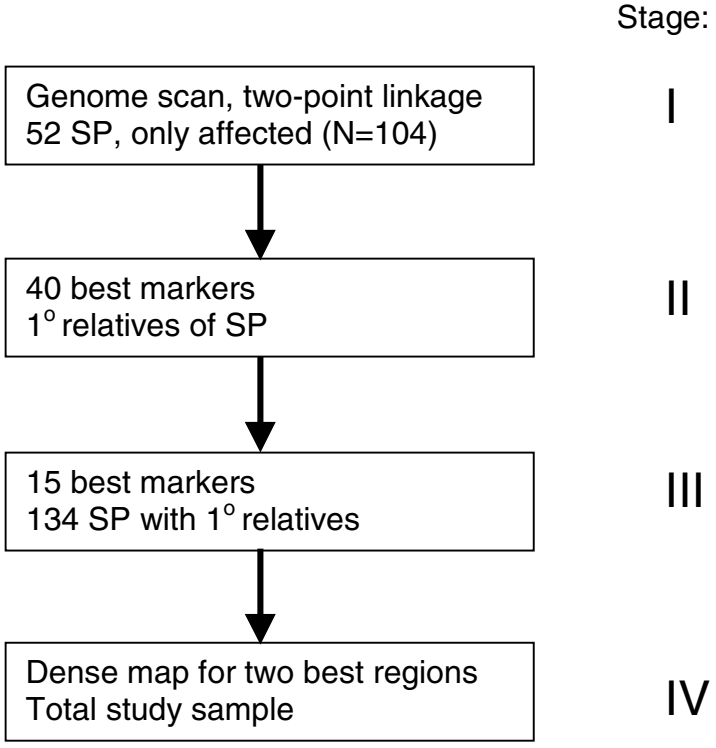
RESULTS AND DISCUSSION

1 Genome-wide scan in sib-pair families from Finland

A schematic view of the general design of the study is given in Figure 8. To optimize both cost-effectiveness and our chances of finding true positive linkages, the study was performed in four stages, so that additional individuals were genotyped or markers added at each stage based on the findings of the previous stage.

In stage I, we genotyped 370 polymorphic microsatellite markers from the Weber screening set 6 (Sheffield et al. 1995) in 52 affected sib-pairs (104 individuals) without parents. Thirteen markers resulted in lod scores over 0.5 and three markers produced Z_{max}>1.0. The highest pairwise lod scores were found for markers D10S2325 (ASP Z_{max}=1.87), D5S1473 (ASP Z_{max}=1.46), and D14S610 (ASP Z_{max}=1.49). On the basis of stage I data we selected markers to be included in stage II.

Figure 8. Schematic overview of the four stages of study I.
(SP = Sib-pairs)



In stage II we genotyped the 40 markers providing the highest lod scores in stage I in DNA samples of parents or unaffected siblings of the affected sib-pairs. This stage was expected to decrease the likelihood of false positives, and to increase the ability to detect genotyping errors that might lead to false negatives (Terwilliger et al. 1992), providing a more reliable basis for selection of markers to be analyzed in the final stages.

In stage II, 23 markers resulted in affected sib-pair lod scores higher than 0.5, ten of which were higher than 1.0. The markers providing the highest ASP lod scores were D5S1473 (Z_{max}= 1.61) and D17S122 (Z_{max}= 1.60). For stage III we selected the best 15 markers based on the data from stage II.

In stage III, we genotyped an additional set of affected sib-pairs and their first-degree relatives, resulting in a total of 134 affected sib-pairs. Based on the linkage analysis of the 15 markers included in this stage the two most interesting regions were selected for further study in Stage IV.

In stage IV we genotyped 29 markers in the two chromosomal regions providing the highest stage III ASP lod scores. These were a 16 cM region around marker D7S1799 which resulted in the highest lod score in stage III (ASP Z_{max}=2.42) and a 30 cM region around markers D1S1656 (ASP Z_{max}=1.72 in stage III) and D1S2141, for which evidence of linkage was found in a previous genome scan performed in the study sample from a sub-isolate of Finland (Hovatta et al. 1999).

On chromosome 7, the region between markers D7S477 and D7S486 resulted in two-point lod scores up to 2.54 (D7S1799) under liability class 3. A broad peak was observed between the same markers in multipoint analysis using the MAPMAKER/SIBS 0.9 program (Kruglyak and Lander 1995). The highest MLS, 2.62, occurred close to marker D7S1799.

We then incorporated the genealogical data collected for the families into the statistical analyses. When only the families originating from the late settlement region of Finland (Norio et al. 1973) were included in the analysis, the obtained lod scores were higher. Marker D7S486 produced a two-point lod score of 3.18 for the dominant model under liability class 3. Multipoint analysis using MAPMAKER/SIBS resulted in a MLS of 3.53 between markers D7S501 and D7S523, under the broadest diagnostic model (class 4).

On chromosome 1 we identified a positive region between markers D1S439 and D1S1656, about 4.1 cM wide on the genetic map, which resulted in lod scores up to 2.62 under the broadest diagnostic model and dominant mode of inheritance. The same region was also implicated by multipoint analysis with MAPMAKER/SIBS with a MLS of 2.51, between markers D1S439 and D1S251.

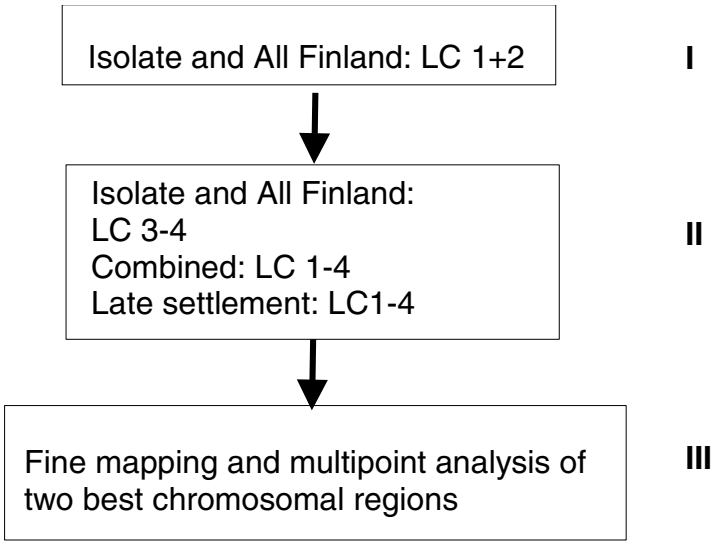
This study replicated the previous finding by Hovatta et al. (1999) performed on a study sample from a genetically isolated region in northeastern Finland. The markers providing the strongest evidence for linkage on chromosome 1 in these two studies are about 15 cM apart on the genetic map, well within the limits of expected variation of the lod score peak in study samples like these (Roberts et al. 1999). The major problem with this study was that since only affected siblings were identified in the first stage, false negative findings are more likely, especially since not all genotype errors having a detrimental effect on the allele sharing statistic could not be detected. The linkage to the chromosome 7 region was novel and has not been replicated by other groups. This finding is interesting, since one gene independently suggested as a candidate gene for schizophrenia (RELN) is located in this region (Fatemi 2001), along with several other interesting candidate genes, e.g. Semaphorin-3A (SEMA-3A). Interestingly, there is strong evidence for the involvement of a gene on this chromosomal region in autism (IMGSAC 2001a, b). Even though they are distinct diagnostic entities, autism and schizophrenia share some common features, specifically the apparent social anhedonia can be thought of as an overlapping symptom. In the future we will learn whether autism and schizophrenia share any part of their genetic background on chromosome 7q. The linkage in the chromosome 1 region has been replicated by several groups and also contains one of the strongest candidate genes for schizophrenia to date (DISC-1) (Millar et al. 2000). This region is discussed more in detail in connection with study III.

2 Genome-wide scan in a population-wide sample from Finland

The sample collection continued during and after Study I. Experiences from genome-wide scans for schizophrenia performed internationally had proven that larger sample sizes were necessary in complex traits. We therefore initiated the genome-wide scan of a study sample considerably larger than in any of our previous studies. Moreover, the whole sample was included in all stages of the study, in contrast to the previous studies that had been staged in the interest of cost-effectiveness. This stage of the study was expected to decrease false negative findings and to expose new chromosomal regions of interest.

In two-point linkage analyses of the 53 pedigrees from the internal isolate, the best evidence for linkage was obtained with marker D2S427 on chromosome 2q37. Using the recessive model of inheritance in nuclear families under the most restricted diagnostic model, a two-point lod score of 4.43 was obtained

Figure 9. Schematic overview of the three stages of Study II (LC = liability class). The whole study sample was used in all stages of the study.



for this marker. Lod scores exceeding two were obtained for markers on chromosomes 1 (D1S1728), 3 (D3S1311), 5 (GATA81C06) and 18 (D18S877).

In two-point linkage analysis of the families from the nationwide sample, excluding the families from the internal isolate, the strongest evidence for linkage , Z_{max} = 3.16, was obtained for marker D5S820 using liability class 1 and the dominant inheritance model. No other marker provided a lod score ≥ 2.0 in the first stage of the analysis.

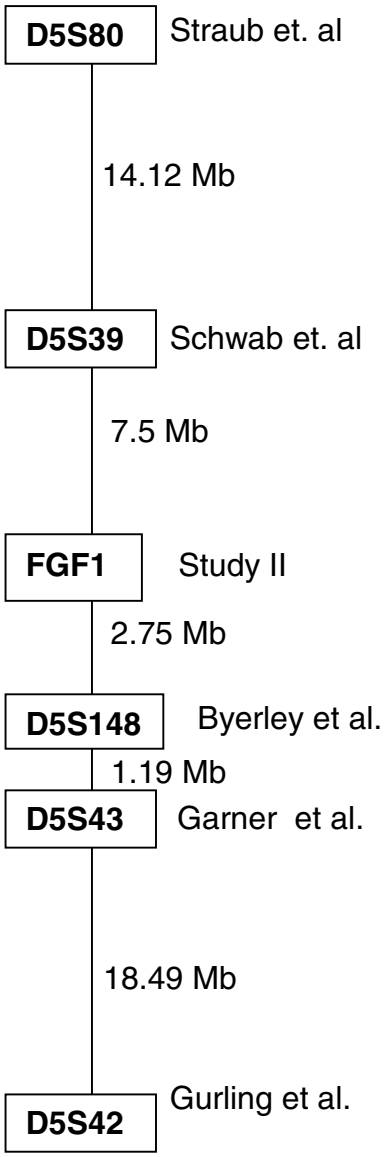
Like in Study I, the sample could be stratified based on the population history of Finland into early and late settlement families. In total, 118 families originating from the late settlement region of the country were analyzed with the 27 markers giving lod scores ≥ 1 in the nationwide sample. Four markers (D1S431, D4S2394, D5S1480 and D12S1294) provided increased evidence for linkage compared to the nationwide sample, the most significant being D5S1480 (Z_{max} = 2.05).

The total study sample consisted of 238 pedigrees and 1,251 genotyped individuals. This study sample was analyzed for the best 62 markers from the analysis of the individual samples. The best evidence of linkage (Z_{max} = 3.55) was obtained for marker D5S820. Marker D2S427 yielded a lod score of 3.30 for liability class 1.

Based on the two-point linkage analyses, two chromosomal regions appeared the most interesting: chromosomes 2q and 5q. We conducted multipoint analyses on these chromosomal regions using SimWalk2. We used only the liability class and analyzed only the study sample that had shown the best evidence for linkage in the two-point analyses in the corresponding region.

Due to its complicated structure and extensive size, the largest pedigree from the internal isolate sample was always analyzed separately from the rest of the pedigrees. On chromosome 2q, the best evidence for linkage was obtained near marker D2S427 for the combined study sample with Statistic A ($-\log_{10}(p) = 2.5$). On chromosome 5q the best evidence for linkage was obtained near marker D5S480 for the late settlement study sample ($-\log_{10}(p) = 2.8$). Based on these results, as well as previous linkage findings in this region (Schwab et al. 1997; Straub et al. 1997), we pursued fine mapping with 30 markers over 14 cM on chromosome 5q. The most significant result was obtained with the families from the late settlement region ($-\log_{10}(p) = 3.7$, Statistic A) near marker FGF1.

Figure 10.
Overview of the linkage findings on chromosome 5q, and their relative positions (in Mb)



In conclusion, this study provided important evidence for linkage for two regions not identified in our earlier studies. The finding on chromosome 2 is completely novel, with no earlier reports of linkage to schizophrenia. The chromosome 5 region, on the other hand, is supported by several other studies (Figure 10), especially noteworthy is one of the first significant linkages found in schizophrenia genetics (Sherrington et al. 1988). A recent report on a genome-wide scan in Icelandic and UK families showed evidence for linkage in the same region (lod = 3.6, D5S422, (Gurling et al. 2001)), although this marker is some 20 cM telomeric of the best marker in our study. A study by Straub et al. also found support for a region some 15-20 cM centromeric of D5S414 in 265 Irish pedigrees (D5S804, lod = 3.04, (Straub et al. 1997)). One study of families from Israel and Germany (Schwab et al. 1997), and another of families from Palau (Byerley et al. 1999) have provided further support for this region. The findings from the present study have a high statistical significance and have been produced using one of the largest study samples collected for genetic studies of schizophrenia to date. Therefore both of them certainly warrant further studies aimed at identifying the polymorphism responsible for the increase in schizophrenia susceptibility contained in the respective regions.

3 Detailed study of chromosome 1 in families with schizophrenia

Chromosome 1q has emerged as one of the major candidate regions for schizophrenia based on several findings. The finding of Hovatta et al. (1999), as well as data from Study I in this thesis made a relatively strong case for linkage of schizophrenia in Finland to chromosome 1q31-42. One genome-wide scan for bipolar disorder gave the second best evidence for linkage in the same region (Detera-Wadleigh et al. 1999), as well as one genome-wide scan for schizophrenia utilizing a study sample from Dagestan (Bulayeva et al. 2000). One finding 60 cM more telomeric to this region also gained much attention (Brzustowicz et al. 2000). The interest increased further when a gene designated as disrupted in Schizophrenia 1 (DISC1) was identified (Millar et al. 2000). A translocation disrupting this gene co-segregates with schizophrenia in a large Scottish pedigree giving a lod score of more than 6. This gene is located within 1-2 cM from the marker giving the strongest evidence for linkage in Study I, making it a strong positional candidate gene also for schizophrenia in the genetically isolated Finnish population. Based on all of these findings, we set out to study chromosome 1q more in detail in order to define the region of interest and to analyze the contribution of different study samples to the linkage evidence.

The results of this study are presented separately for three different study samples: 1) extended families with well established genealogical data from the internal isolate of the late settlement region of Finland, 2) the generally smaller families originating from the rest of Finland (referred to as 'nuclear families' hereafter), and 3) the two study samples combined.

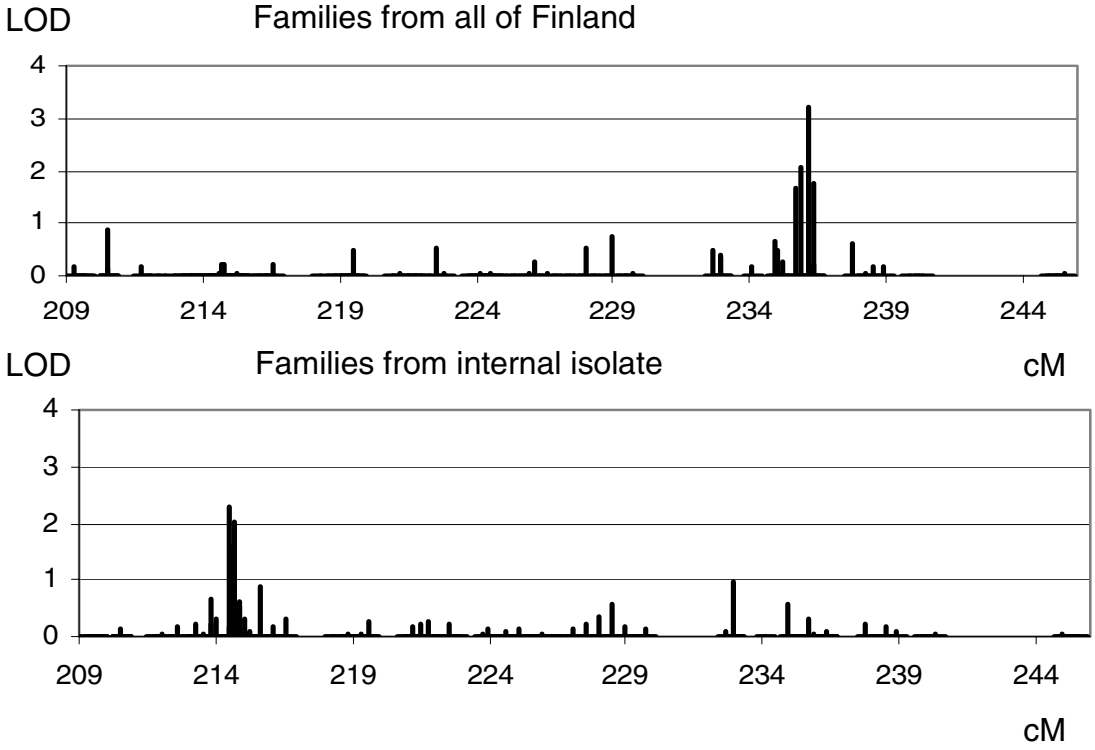
For the combined sample, the highest lod score was obtained for marker D1S2709 (lod = 2.71), adopting the dominant inheritance model and treating individuals with schizophrenia spectrum disorders (liability class 3) as affected. Several markers in a 2 cM region around this marker gave two-point lod scores greater than 1.

For the families from the internal isolate of Finland marker D1S245 gave a lod score of 2.30, again adopting the dominant model classifying all individuals with schizophrenia spectrum disorders as affected (Figure 11). This region is the same as the one identified in the previously published genome-wide scan in this sub-isolate (Hovatta et al. 1999). Evidence for co-segregation was also seen for marker D1S1728 (lod = 2.44, dominant model, liability class 3), which is more than 100 cM proximal of D1S245.

For the nuclear families from the rest of Finland, marker D1S2709 gave the strongest evidence for linkage (lod = 3.21), using a dominant model treating individuals with spectrum diagnoses as affected (Figure 11). This marker is within 3 cM of the peak identified in our previously published sib-pair genome-

wide scan (Ekelund et al. 2000). No evidence for linkage to the position identified in the internal isolate was found in these families.

Figure 11. Two-point linkage results for familes from the internal isolate and the rest of Finland, respectively.



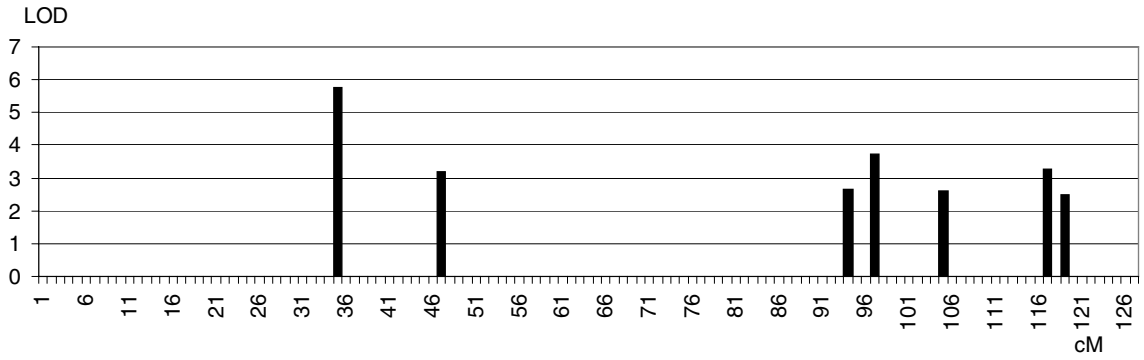
The gamete competition model showed results significant at the 5% level for 17% of the markers, while this would have been expected of only 4% of the markers by chance based on a gene-dropping simulation. The strongest evidence for association and linkage for the combined study sample was seen for marker D1S225 (p=0.009). The evidence for association and linkage to this marker emerged exclusively from the nuclear families (p=0.005), while the families from the internal isolate showed no evidence of association. This marker is within 1 cM of marker D1S2709, which gave the strongest evidence for linkage in this study.

One of the markers we genotyped (D1S484) is within 2 cM from the marker that gave the strongest evidence for linkage to schizophrenia in a recent study performed in Canadian families (Brzustowicz et al. 2000). This particular marker provided only very weak evidence for linkage (lod=0.88) in our study.

For the 10 markers around D1S245 that showed evidence for linkage in the two-point analysis of the families from the internal isolate, statistic B of SimWalk2 gave the strongest evidence for linkage close to marker D1S245 ($-\log_{10}(p)=3.46$) in families from the internal isolate. For this region the nuclear families from the rest of Finland did not show evidence for linkage. The largest pedigree from the internal isolate, with 31 affected family members, provided

the majority of the statistical evidence for linkage, while the rest of the families from this restricted geographical area contributed very little. For the 10 markers flanking marker D1S2709, which gave the strongest evidence for linkage for the total study sample in the two-point analysis, statistic B of SimWalk2 showed the strongest evidence for linkage close to marker D1S2709 ($-\log_{10}(p)=1.94$). The evidence for linkage was obtained almost exclusively from the nuclear families.

Figure 12. Overview of linkage findings on chromosome 1 in studies of schizophrenia and bipolar disorder. From the left, the bars represent the maximum lod scores obtained by: Brzustowicz et al. (2000) - Gurling et al. (2001) - Detera-Wadleigh et al. (1999) - Hovatta et al. (1999) - Ekelund et al. (2000) - Ekelund et al. (2001) - Millar et al. (2000)



This part of the study did not provide any significantly stronger evidence for linkage than the previous studies, nor were we able to restrict the area of interest much from what was previously known. It is interesting, however, that the results do not change much at all when such a massive amount of data is added. Families from all over Finland were added, but the finding remained roughly the same. This makes it likely that the possible susceptibility gene contained in this chromosomal region has an effect on schizophrenia susceptibility in families from all geographical areas of Finland. We cannot with certainty tell whether the two linkage peaks observed in the two sub-samples truly reflect two separate susceptibility loci or whether they merely reflect stochastic variation in the localization of the lod score peak in different samples. As can be seen from Figure 12, a substantial amount of evidence for linkage to chromosome 1q has been published recently. All findings taken together, it is likely that chromosome 1q contains at least one gene that has a detectable effect on schizophrenia susceptibility. Several groups are presently investigating this region to identify and characterize the putative psychosis gene.

4 The incidence of cancer in patients with schizophrenia and their first-degree relatives

The multicenter WHO collaborative study of cancer incidence in schizophrenic study samples showed that the incidence of lung cancer in particular was decreased, but left the reasons behind this counterintuitive finding unexplored (Gulbinat et al. 1992). To take this matter one step further we used the extensive and relatively complete registers available in Finland to verify this finding and also to study the incidence in the first-degree relatives of the schizophrenic patients to explore familial-environmental hypotheses about this paradox.

After exclusion of 698 individuals who had died before the starting date of the follow-up, 26,996 patients with schizophrenia, 52,976 of their non-schizophrenic siblings, and 39,131 of their parents were eligible for follow-up. This corresponded to 446,653, 1,438,143 and 905,947 accumulated person years at risk of cancer (or a mean of 16.5, 27.1, and 23.2 years at risk per subject) respectively. Of all the cases reported to the Finnish Cancer Registry, 1,533 were benign lesions (mainly basal cell carcinomas of the skin) and thus excluded.

Among patients with schizophrenia, 724 cancers were diagnosed during follow-up while 619 would have been expected from the incidence in the general population. The overall risk of cancer was significantly increased (SIR 1.17; 95% CI, 1.09-1.25), more so in males than females. The largest increases were found for primary cancer of the lung (SIR 2.17; 95% CI, 1.78-2.60) and pharynx (SIR 2.60; 95% CI, 1.25-4.77). A significant increase was also seen for cancer of the gall bladder (SIR 2.07; 95% CI, 1.03-3.70), the SIR being higher in males (SIR 3.01; 95% CI, 0.98-7.01) than in females (SIR 1.64; 95% CI, 0.60-3.57). The SIR for rectum (SIR 0.35; 95% CI, 0.13-0.75) cancer was decreased and that for cancer of the corpus uteri increased (SIR 1.75; 95% CI, 1.19-2.48).

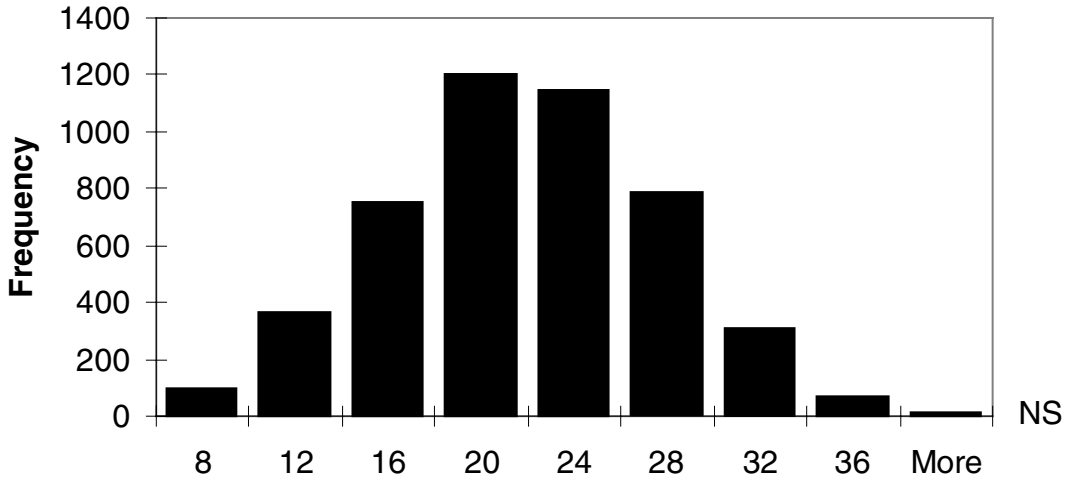
Among non-schizophrenic siblings and parents of schizophrenia patients fewer cases of cancer emerged than expected (siblings: 886 vs. 999; SIR 0.89; 95% CI, 0.83-0.94; parents: 6,165 vs. 6,755; SIR 0.91; 95% CI, 0.89-0.93), similarly in both sexes. Decreased incidences of lung cancer (SIR 0.82; 95% CI, 0.68-0.97) and breast cancer (SIR 0.77; 95% CI, 0.71-0.83) were seen in patients' mothers but not in other relatives. Cancer of the corpus uteri occurred less frequently than expected both in mothers (SIR 0.81; 95% CI, 0.69-0.92) and sisters (SIR 0.38; 95% CI, 0.14-0.83). Prostate cancer in patients' fathers also occurred at a lower rate, though the 95% CI included unity (SIR 0.57; 95% CI, 0.26-1.08). The incidence of colon cancer in parents was lower than expected (291 vs. 347; SIR 0.84; 95% CI, 0.75-0.93).

Based on these results we can conclude that cancer incidence is really increased among Finnish individuals with schizophrenia and decreased among their first-degree relatives compared to the general population. The finding of increased incidence among the schizophrenic subjects is consistent with what is known about the smoking prevalence and intensity in this group of patients. As a matter of fact, the relative increase in cancer incidence is not statistically different from what would be expected based on the smoking data available, even though data on smoking was not available on the individual level (Jablensky and Lawrence 2001). The finding in the relatives is not as readily understandable. Some factor obviously decreases one's risk of being diagnosed with cancer if one has a first-degree relative diagnosed with schizophrenia. It is unclear what this factor could be, but both environmental and genetic factors are conceivable. One possible environmental factor is relatives becoming more health-conscious when their sibling or child develops schizophrenia and consequently being less exposed to carcinogenic agents. This seems unlikely to explain a large deviation in cancer risk among relatives, but cannot be ruled out. A completely hypothetical genetic risk factor could decrease the risk of malignancy while increasing the risk of developing schizophrenia. The protective effect of such a gene would be seen among first-degree relatives of schizophrenic patients since they share on average half their genome with the patients, but could be overridden in the patients by their excessive use of tobacco and alcohol, among other risk factors.

5 The association between novelty seeking and DRD4

The exponential rise in risk of schizophrenia with increasing degree of genetic relationship indicates the importance of non-linear interactions among multiple genetic factors (Risch 1990b). If it holds true that some complex disorders, like schizophrenia, are caused by interactions among several susceptibility dimensions, each of which may be oligogenic, replication of particular genes is unlikely using the sample sizes that are possible to collect (Risch 1990a). The idea behind this study was to identify genes contributing to temperament, which has a relatively simple genetic architecture. If such genes are identified, susceptibility to e.g. schizophrenia could be evaluated in terms of risk from heritable temperament measures and disease-specific factors. The total allele frequencies in our sample were similar to the allele frequencies in a Finnish control population published earlier (Malhotra et al. 1996). The distribution of the NS scores is shown in Figure 13. The mean score was 20.3 (95% confidence interval for the mean 20.2-20.5, SD = 5.9). All the subjects included in the analysis had NS scores more than ±1 SD from the mean of the cohort (NS score in low scorers ≤ 12, in high scorers ≥ 31) and the means for the extreme groups (7.48 and 33.48 respectively) lay outside ±1.96 SD from the mean for the whole group.

Figure 13. The distribution of Novelty Seeking (NS) scores in the Oulu66 birth cohort.



Contrary to the original finding (Benjamin et al. 1996; Ebstein et al. 1996) the 7 allele was less common among our high scorers, whereas the 2 and 5 repeat alleles were more frequent in this group. One subject had an 8-repeat allele, and this was pooled with the 7-repeat allele group in all analyses. For 10 individuals DNA did not amplify satisfactorily and they were excluded in all analyses.

The value for the Pearson Chi-Square statistic was 14.1, which for 4 degrees of freedom corresponds to a global p-value of 0.007 for both sexes together over the contingency table. The sex-specific analysis using the chi-square statistic was hindered by too small expected values for some of the cells. However, a trend in the same direction as for the sample as a whole was observed. When allele 5 was pooled with the allele closest to it in size (allele 4) there was a non-significant trend in the same direction as for the whole sample (males: $\chi^2 = 4.1$, 3 df, $p = 0.248$; females: $\chi^2 = 5.8$, 3 df, $p = 0.123$).

As can be seen from the above, the association we observed was in the opposite direction compared to the original findings, that is the 7-repeat allele was less common among high NS scorers in our sample than among low scorers, and the opposite holds true for the shorter alleles (2- and 5-repeat alleles). There are several explanations for this discrepancy. First of all, both findings might naturally be false positive, a possibility that can only be ruled out by further, independent studies of this issue. Secondly, our study sample might in some important way differ from the original reports (Benjamin et al. 1996; Ebstein et al. 1996). For example, since we included only extreme NS scorers we might actually have included a larger proportion of subjects with any diagnosable personality disorder or other mental disorder. One might speculate therefore that the finding in our study is primarily related to any such disorder and only secondarily to Novelty Seeking (Malhotra and Goldman 2000). Thirdly, it has been postulated that there could be a non-

linear relationship between a quantitative trait and frequency of a certain variant of a gene that truly has an effect on that trait. This has been shown to be the case for example between neuroticism and a polymorphism in the promoter of the serotonin gene (Sirota et al. 1999). If this is also the case for NS and DRD4, our results are difficult to interpret due to our sampling strategy. The last and probably most plausible explanation is that the studied polymorphism is actually not the one influencing NS scores, but that it is merely in linkage disequilibrium with the true, causative variant. Another variant, namely a -521 C/T polymorphism in the promoter region of DRD4, has also been found to be associated with NS scores (Okuyama et al. 2000). In our sample however, this specific polymorphism was not associated with NS scores (Ekelund et al. 2001). It is therefore possible that yet another polymorphism in the DRD4 gene, its regulatory region or in a gene closely situated on the chromosome is actually the one influencing NS scores. Taken together, these results replicate the original findings of an association between NS and DRD4. If this association holds true, it is the first finding of a gene that has an effect on variation in a normal behavioral trait. Identification of genes for normal personality may shed some light on the etiology of psychopathology as well.

CONCLUDING REMARKS AND FUTURE PROSPECTS

This study began in the spring of 1996, since when much has happened in the field of genetics, and specifically in the field of psychiatric genetics. Dense marker maps and high-throughput genotyping techniques have replaced the earlier sparse maps and labor-intensive genotyping methods. In 2001, moreover, the first draft of the sequence of the human genome was published, a major milestone in biological and medical research.

Some less welcome insights in psychiatric genetics have emerged during this time. While many involved scientists in 1996 believed that a major gene for schizophrenia, or any other major mental illness, would soon be identified, we have now learned that such major genes probably do not exist and that most common psychiatric disorders are genetically complex and heterogeneous. This has influenced the way scientists conduct genetic studies. Less than ten years ago many studies were published that reported linkage in one or only a few pedigrees. Studies of this type have now been replaced by massive efforts by international research institutions, both academic and commercial, to pool study samples from multiple populations. The future will hopefully and probably see even bigger collaborations aimed at achieving sample sizes sufficiently large also to detect genes of minor effect.

1996 saw the first publication describing a genetic variant affecting normal human behavior. This finding, if it holds true, will certainly have an effect on how we view ourselves as human beings. Spurred by this original finding much research is now being conducted on normal variation in behavior. In the future, findings of this kind will tell us a lot about how the human brain works and also what goes astray when some parts of the brain function abnormally, resulting in psychiatric disorders.

The present study has made contributions to three areas of research: 1) The search for susceptibility genes for schizophrenia, 2) The genetics of normal human behavior, 3) The comorbidity of schizophrenia. While these contributions have naturally been small, they have still expanded our knowledge of the genetics of psychiatric diseases in Finland.

During the past few years the importance of the diagnostic process in genetic studies on mental disorders has, if anything, increased. Not only is it necessary to use diagnostic systems with high reliability, but developing new diagnostic categories might also be a vital prerequisite to identify susceptibility genes for any psychiatric disorder. It is possible that the present practice of using clinically based diagnostic systems might not prove valid enough from a pathophysiological perspective. These systems have high reliability, a feature necessary for clinical use, but might not define pathophysiologically distinct entities. It might be necessary to measure more fundamental quantitative

traits that have a closer relationship to the underlying pathophysiology. Therefore development of radiological, neurophysiological and neuropsychological measures that reliably and significantly define some sub-population of schizophrenic patients might be necessary before we can get a firm grasp on the genetic background of schizophrenia.

Much work will be required in the fields of bioinformatics and statistics. We need biocomputing tools capable of handling much larger amounts of data than are presently available, and also statistical tools able to manage completely new kinds of data. We will need to be able to determine genome-wide genetic profiles, to identify gene families and to predict function from structure. New techniques monitoring expression profiles and interaction between genes will require new biocomputing tools. In addition, we will need statistical methods to analyze larger amounts of data - both genotype and genealogical - and to control for numerous quantitative, epistatic and other complex effects of human genes.

The new findings in genetics, especially complex genetics, have also had some effect on the opinions and views of the public, and hopefully this influence will increase. The common view of "genetic" disorders has largely been influenced by the early findings of causal connections between mutations and disorders in the rare, monogenic disorders. We now know that most common disorders are genetically complex, with both genetic and environmental factors having an effect on the onset and outcome of the disorder. Therefore most disorders are not "genetic" or "environmental"; they are both. Hopefully, this insight will start to shift common views of genetics away from fatalistic determinism and towards a heuristic optimism that much is still to be learned about the etiology, pathophysiology and potential interventions of common disorders, and specifically mental disorders, based on their genetic background.

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I am most privileged to have had two supervisors who are both distinguished authorities in their respective fields of research. Professor Leena Peltonen-Palotie's dynamic leadership has ensured that the overall project continually evolves. Professor Jouko Lönnqvist's knowledge and understanding of the complicated field of psychiatry are, along with his leadership skills, truly admirable. I especially want to thank both my supervisors for creating an atmosphere of mutual trust and respect that is really quite flattering at the beginning of one's scientific career.

I wish to thank professor Jari Tiihonen for accepting the role as custos in my defense.

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When I first arrived in the lab, I was warmly welcomed by Drs. Iiris Hovatta and Dirk Lichtermann. With incredible patience Iiris taught me virtually everything I know about lab work and also introduced me to linkage analysis. I am greatly indebted to her. Dirk, a living textbook of psychiatry and psychiatric epidemiology, proved invaluable as I learned the basics, and still continues to be an inspiring collaborator. I also had many fruitful discussions with Dr. Petra Pekkarinen about the difficulties of complex trait mapping.

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When investigating a disorder purely defined by its symptoms, the reliability and accuracy of diagnoses is naturally crucial. I want therefore to thank all the people who have read countless pages of patient records: Drs. Jaana Suvisaari, Hannu Juvonen, Ritva Arajärvi, Marja-Liisa Kokko-Sahin, Jaana Suokas, Maria Muhonen and Taru Mäkikyrö, as well as Timo Partonen who has coordinated this process. The fact that they are all doing related, high-quality research guarantees that the diagnostic process is the best it can be. The neuropsychologist of the project, Ammi Tuulio-Henriksson, has brought the science of quantitative genetics into the project by studying QTs related to schizophrenia. Antti Tanskanen and Marjut Schreck have provided us with the accurate data we use in our analyses.

I also want to thank the data-collection team, without whom we literally would have nothing to study. Liisa Moilanen, a wizard of blood sample collection, proved invaluable during the early years of the study. Many others later joined the team to further hasten the collection not only of blood samples but also phenotypic data.

The productivity of the project over the last few years has been boosted by scientific collaboration with Millennium Pharmaceuticals Inc. Dr. Alex Parker and his team including Alessandro Foti and Gayun Chan have produced genotypes at an amazing rate. Dr. Rory Martin has made valuable contributions not only to the analysis of the data, but also to strategic decision making. I also want to thank Alex and Rory for their valuable comments on various manuscripts. The team lead by Dr. Steve Lewitzky has been very efficient in SNP discovery. The work of Sanchit Maruti and Kate Rinard has made the databases of Millennium and our institute compatible. Jennifer Morgan is acknowledged for keeping track of the whole project and also for taking good care of us during our visits to Boston. Dr. Joanne Meyer has been leading the group at Millennium, and our discussions on different approaches

to identifying genes for complex disorders have proved very rewarding. Dr. Charles Richard, the representative of Wyeth-Ayerst Research in most of our meetings, is acknowledged for his positive attitude towards this project, and for his genuine informed interest in its progress at all levels.

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The somewhat chaotic, yet productive atmosphere at the Department of Human Molecular Genetics (later Molecular Medicine) has been a continuous source of inspiration. Most importantly, my friendship with Ville extends far beyond the lab. The "grappa frizzante della Sestri Levante" is an evergreen excuse for throwing a party, thanks to Emma, Ilona, Jenni and Jr. Nikke. Jenny is great company in the "psycho" room and Tintti makes the coffee breaks more cheerful. Markus has always been very helpful and has taught me much about complex genetics. Tomi has been very useful with the technical stuff. Lasse has been good company and an invaluable consultant during my short period as a GP. The computer/bioinformatics guys, Aki, Tero, Teemu and Jari, have all been vital and always helpful, despite the constant competition for their attention from others. I have learned a lot through discussions with the other "complex mappers" of our group: Päivi P, Satu, Miina, Mari A, Tero and Janna. Sari, Sari, Tuula and Tiina are thanked for their great help in everyday research matters.

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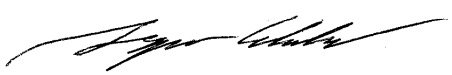

Helsinki, November 6th, 2001 Jesper Ekelund

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