Diagnostic Evaluation of Schizophrenia for Genetic Studies

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Umeå 2005
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Published and printed by Print & Media Umeå, Sweden: 2001239
To Mattias and Frida

The mind is its own place, and in itself
Can make a Heav’n of Hell, a Hell of Heav’n
- John Milton, Paradise Lost
ABSTRACT

Background: Schizophrenia is one of the top-ten leading disorders causing disability worldwide. Heredity is accepted as a major causative factor but the mechanisms are still unknown. Molecular genetic studies have resulted in contradictory results. To find molecular mechanisms behind schizophrenia, patient materials with reliable valid diagnoses must be identified. The aims of the present thesis were: 1) to recruit Swedish patient materials with a conceivable diagnoses of schizophrenia and to certify these diagnoses. 2) to compare schizophrenia diagnostic procedures for reliability, validity and suitability for genetic studies by evaluation of record information, interview data and national register diagnostic data. 3) to examine the patient materials for linkage or association with molecular genetic markers. Three patient materials with schizophrenia were recruited, sporadic cases, a large pedigree with schizophrenia and Swedish sib-pairs.

Results: Schizophrenia research diagnoses based only on patient records showed good to excellent agreement with diagnoses based on both records and interviews. Register diagnoses generally displayed poor agreement with research diagnoses, but in 94% of patients sometimes registered as schizophrenic psychoses (i.e. schizophrenia, schizoaffective psychosis or schizophreniform disorder) a research diagnoses of these disorders were certified. In the pedigree, analysis focusing on the short arm of chr 6 suggested linkage to 6p23 in a single branch of the pedigree, indicating heterogeneity within the family. In the same pedigree a whole genome scan indicated linkage to the 6q25 region. A whole genome scan analysis of the Swedish sib-pair material was suggestive of linkage to chr 10q25.3-q26.3. In the case-control sample there was an association between a putative functional dopamine D2 receptor polymorphism (Ser311Cys), on chr 11q22-23, and the disorder. This finding was apparent also in a meta-analysis of published studies. In the same patient material several brain-derived neurotrophic factor gene variants (chr 11p13) were also analysed without any robust significant findings.

Conclusions: For patients in long-term treatment for schizophrenia in Sweden, psychiatric record reviews should be valid, reliable and sufficient for assessment of lifetime research diagnosis of schizophrenia. A structured interview adds little new information. Swedish register diagnoses of schizophrenic psychoses have a high positive predictive power in relation to research diagnoses of these disorders. For future Swedish large-scale genetic studies focusing on a broad definition of schizophrenia, it is sufficient to rely on the register diagnoses of schizophrenic psychosis. There is no major vulnerability gene or locus that is common to the majority of patients with a current research diagnosis of schizophrenia. The results indicate a substantial heterogeneity with regard to actions of a number of genes with small effects. It is also questioned whether the use of the syndromal concept of schizophrenia as a research diagnosis is useful. To find the molecular genetic bases behind psychotic disorders, analyses of specific genetic signs and symptoms and other phenotypic features of the patients may be more meaningful.
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LIST OF ABBREVIATIONS

ASP  Affected sib-pair
Chr  Chromosome
DIGS  Diagnostic Interview for Genetic Studies
DNA  Deoxyribonucleic acid
DSM  Diagnostic and Statistical Manual
DZ  Dizygotic
FIGS  Family Interview for Genetic Studies
HUBIN  Human Brain Informatics
ICD  International Classification of Diseases
LOD  Logarithm of odds ratio
MZ  Monozygotic
OPCRIT  Operational Criteria Checklist
p  Short arm of a chromosome
PCR  Polymerase chain reaction
q  Long arm of a chromosome
R  Record
R+I  Record and Interview
SAPS  Scale for Assessment of Positive Symptoms
SANS  Scale for Assessment of Negative Symptoms
SCAN  Schedules for Clinical Assessment in Neuropsychiatry
SCID  Structured Clinical Interview for DSM
SNP  Single Nucleotide Polymorphism
SRD  Standard Research Diagnosis
SPIR  Swedish psychiatric inpatient register
1 INTRODUCTION
1.1 THE CONCEPT OF SCHIZOPHRENIA

Madness is probably as old as humankind, and mental disturbances have been described for at least four thousand years. The concept of schizophrenia has fascinated and puzzled researchers and laymen for almost a hundred years. Emil Kraepelin described patients with certain symptoms now associated with the diagnosis of schizophrenia. He coined the term dementia praecox for these patients. The onset was in early adult life with progressive deterioration. The concept of schizophrenia (from Greek; schiz split, phren mind) is less than 100 years old and was defined by the Swiss psychiatrist Eugen Bleuler. He based this diagnosis on the signs and symptoms he considered common to a number of patients. Bleuler distinguished four primary symptoms, i.e. association disturbance, affective disturbance, ambivalence and autism. In Bleuler’s view the splitting of mental functions was the main feature of patients with schizophrenia. Delusions and hallucinations were also common but these he considered these to be secondary to the primary symptoms. The symptoms usually appeared in young previously healthy individuals. Bleuler regarded schizophrenia as a group of disorders and the course was not necessarily deleterious. Kraepelin’s theories have markedly influenced European and Swedish psychiatry. Bleuler’s views have dominated psychiatry in the USA. Based on Kraepelin’s and Bleuler’s traditions, an extensive literature has been produced to further explore the characteristics of schizophrenia. There are several important Swedish contributions to schizophrenia research. One of them, Torsten Sjögren published a classical description of patients with schizophrenia exhibiting symptoms as ambivalence, negativism, association disturbance, affective splitting, etc.

Symptoms of schizophrenia have also been sub-grouped into two main categories, positive and negative. Reality distortion, delusions, hallucinations, disorganisation, thought form disorders, inappropriate affect
and bizarre behaviour are considered positive symptoms. Negative symptoms include psychomotor poverty, poverty of speech, blunted affect, and decreased spontaneous movement. The positive symptoms are easier to recognise and measure than the negative symptoms\textsuperscript{10, 99}. The latter are more persistent, cause more disability and are more important for prognosis\textsuperscript{93}. Positive and negative symptoms can also occur in other neuropsychiatric and somatic conditions. Therefore a differential diagnostic attitude is important. It can be questioned if any specific symptom is pathognomonic for schizophrenia\textsuperscript{29}.

During the last few years, several authors have refocused on Kraepelin’s view of patients showing cognitive impairment. It has been proposed that abnormal cognitive functioning is a possible causal risk factor for psychosis, representing a third group of symptoms\textsuperscript{155}. Cognitive impairment comprises deficits in abstraction, verbal memory, vigilance, language and executive functions\textsuperscript{9, 22, 79}. There is an ongoing discussion about the cognitive deficits. In particular, it has been debated whether cognitive deficits can represent a core feature of the disorder and a major contributing factor to the poor outcome.

The clinical symptoms are still the basis for the psychiatric diagnoses, because valid biological markers have not been found so far. The signs and symptoms of schizophrenia vary from patient to patient as regards character, intensity and frequency. No single symptom is unique to schizophrenia. There is also a marked variability in premorbid adjustment, type of onset, course and outcome. The clinical heterogeneity of the illness indicates that schizophrenia may include a number of different disorders, each reflecting a specific pathogenic process already indicated by Bleuler’s title “Die Gruppe der Schizophrenien”\textsuperscript{20, 107, 126, 151}.

Schizophrenia usually starts in young adulthood with serious disturbances in thinking, perception and emotions. Life expectancy is reduced by approximately 10 years, mostly as a consequence of suicide\textsuperscript{23, 92, 145, 222}. There
is also a considerable burden for the relatives. Thus the Global Burden of Disease Study\textsuperscript{139} indicates that schizophrenia accounts for 1.1% of the total disability-adjusted life years (DALY) and 2.8% of years lived with disability\textsuperscript{158}. In the World Health Report, schizophrenia is listed as the 8th leading cause of DALYs worldwide in the age group 15-44 years\textsuperscript{5}.

The dichotomy distinguishing schizophrenia from affective disorders has been questioned, mainly because of the difficulties of defining clear and valid borders between the syndromes. It has been argued that instead of two distinct disorders there is rather a continuum of psychosis\textsuperscript{44}. Many individuals with severe psychiatric illness have both mood and psychotic symptoms, raising the possibility that there is not a clear biological distinction between schizophrenia and bipolar affective disorder\textsuperscript{40}. The results from several family studies\textsuperscript{150, 190, 191} are consistent with the existence of a schizophrenia spectrum. The Roscommon Family Study\textsuperscript{103} identified five disorders, within the same spectrum. These were schizophrenia, schizoaffective disorder, schizotypal/paranoid personality disorder, other non-affective psychoses, and psychotic affective illness. The pattern of schizophrenia and related disorders in patients and their relatives may be explained by the same underlying continuum of liability to the "schizophrenia spectrum" and by vulnerability being strongly transmitted within families. Other studies have also reported depression as a possible disorder within the spectrum\textsuperscript{123}. The traditional concept of schizophrenia as a homogeneous disease entity has accordingly become questioned\textsuperscript{152, 195}.

1.2 DIAGNOSIS AND CLASSIFICATION

To obtain international uniformity for schizophrenia research, diagnostic criteria as well as instruments for the assessment of mental disorders have been developed\textsuperscript{65, 176}. Standardized diagnostic criteria were adapted by the American Psychiatric Association when their Diagnostic and Statistical of Manual of Mental Disorders, third edition (DSM-III)\textsuperscript{2} was introduced in 1980.
It was followed by the revised third edition (DSM-IIIR) \(^3\) in 1987 and seven years later by the by fourth edition, DSM-IV \(^4\) (table 1).

**TABLE 1. CRITERIA FOR SCHIZOPHRENIA ACCORDING TO DSM IV**

<table>
<thead>
<tr>
<th>A. Characteristic symptoms</th>
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<tr>
<td>Two or more of the following, each present for a significant portion of time during a one-month period:</td>
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<tr>
<td>• Delusions</td>
</tr>
<tr>
<td>• Hallucinations</td>
</tr>
<tr>
<td>• Disorganised speech (e.g., frequent derailment or incoherence)</td>
</tr>
<tr>
<td>• Grossly disorganised or catatonic behaviour</td>
</tr>
<tr>
<td>• Negative symptoms (i.e., affective flattening, alogia, or avolition).</td>
</tr>
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(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 behaviour or thoughts, or two or more voices conversing with each other.)

**B. Social/occupational dysfunction**

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 previously achieved.

**C. Duration**

Continuous signs of the disturbance persist for at least six months. This six-month period must include at least one month of symptoms (or less if successfully treated) that meet Criterion A.

**D. Exclusion**

Schizoaffective disorder and mood disorder with psychotic features.

**E. Substance/general medical condition exclusion**

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

**F. Relationship to a pervasive developmental disorder:**

If there is a history of autistic disorder or another pervasive development disorder, the diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month (or less if successfully treated).

The development of reliable and cross-culturally applicable diagnostic criteria and instruments for the assessment of mental disorders has also been one of the major goals of the World Health Organisation’s (WHO) mental health programme. In a WHO study, major differences in the incidence of schizophrenia among ten countries disappeared when narrow, standardized diagnostic criteria were used \(^92\). Standardized criteria have also been adapted in the International Classification of Diseases, Tenth revision (ICD-10) \(^221\). The
two diagnostic systems, i.e. DSM-IV (table 1) and ICD-10 are currently the dominating systems used for schizophrenia research. The main difference between DSM-IV and ICD-10 is the duration of certain symptoms before schizophrenia can be diagnosed. In both systems, the occurrence of psychotic symptoms as listed above (table 1), has to be present for a significant proportion of one month. To obtain the diagnosis of schizophrenia according to DSM-IV, but not ICD-10, six months of social/occupational disturbance is also required.

1.2.1 The polydiagnostic approach
Modern explicit criteria have greatly improved the reliability of diagnosis. However, there are several operational definitions of schizophrenia. From the viewpoint of genetic and other biological research there is no clear indication of which one of these that are the most valid. Changes in diagnostic criteria have a degree of randomness and reflex fashion and opinion as much as empirical evidence. An alternative solution is to adapt a polydiagnostic approach, where multiple sets of criteria are applied to the same patients. One tool assigned to this viewpoint, whereby research data are collected in such a way as to allow the application of competing definitions of disorder, is the Operation Criteria Checklist (OPCRIT). It was originally designed to facilitate a poly-diagnostic approach to the diagnoses of psychotic illness and consists of a checklist of symptoms and signs constructed from operational criteria for the major psychiatric classifications. A suite of computer programs generates diagnoses according to 13 different classification systems. Instead of determining whether a patient “meets the criteria”, different symptoms and signs are evaluated by "decomposing" diagnostic criteria into their component items. These can then be reassembled using algorithms based on the original criteria or used to generate novel categories or dimensions.
1.3 PROCEDURES FOR DIAGNOSTIC ASSESSMENT

1.3.1 Instruments for assessment of clinical signs and symptoms

The psychiatric interview is the most common method for collection of anamnestic and observable data. In psychiatric practice, there are different reasons for consulting a psychiatrist. The psychiatric emergency consultation is often due to an occurrence of thoughts, feelings, or behaviour that are intolerable for the patient or for others. This is often the case when a patient is hospitalised. The clinician has to acknowledge the distress and concerns from the patients or from significant others. The central component in the consultation is a face-to-face interview with the patient. In clinical practice this is often performed with “unstructured” methods, i.e. the investigator has no written schedule to follow, but is free to ask the questions and observe the signs s/he feels are adequate for each individual patient. Unstructured interviews will substantially vary from investigator to investigator depending on his/her education, experience and personal preferences. In the hands of an experienced clinician this often means an efficient use of time, directly focussing on the most important topics in the consultation. However, an "unstructured” diagnostic evaluation is likely to introduce intra- and inter-rater differences, thus hampering the reliability \(^{132,159}\). On the other hand, even in scientific settings, the unstructured interview has sometimes been considered to be as good as or better than its structured counterparts. When a group of patients were asked to reflect upon the interview methods used, the unstructured interviewing was often preferred, as it allowed them to describe their experiences and expectations in greater detail than in semi-structured interviews \(^{73}\).

A systematic way of collecting and recording of data is necessary for research purposes. Conclusions must be based upon information that has a satisfactory inter-rater reliability \(^{27}\). Therefore, several semi-structured and structured interview instruments have been developed.
The Structured Clinical Interviews for DSM diagnoses (SCID) are semi-structured interviews for making the major DSM-III-R and DSM-IV axis I or axis II diagnoses. Using a decision tree approach, the SCID-I guides the clinician in testing diagnostic axis I hypotheses as the interview is conducted. The output of the SCID-I is a record of the presence or absence of each of the disorders being considered, for current episode (past month) and for lifetime occurrence. Thus, SCID-I results in none, one or several of about 40 DSM-III-R/DSM-IV axis-I diagnoses by using the diagnostic algorithm. This questionnaire has been widely used internationally, and is among the most commonly used psychiatric diagnostic questionnaires in Sweden. SCID-I has been shown to have a good inter-rater reliability with regard to schizophrenia.

Schedules for Clinical Assessment in Neuropsychiatry (SCAN), is a comprehensive set of clinical assessment instruments developed by WHO and the London Institute of Psychiatry. A central aim for developing this instrument was to provide a standardized interview for ICD-10 diagnoses. SCAN covers a wide range of psychotic and non-psychotic symptoms. A computer program (CATEGO-V) has also been developed for standardized scoring from SCAN, giving algorithm-based diagnoses. SCAN has been field tested in 20 centers in 11 countries.

Diagnostic Interview for Genetic Studies (DIGS) is a semi-structured clinical interview constructed for the assessment of major mood and psychotic disorders and their spectrum conditions. DIGS was developed at the National Institute of Mental Health (NIMH) Genetics Initiative. DIGS was designed to be employed by interviewers who exercise significant clinical judgment and who summarize information in narrative form as well as in ratings. A reliability study was carried out for DSM-III-R criteria-based disorders. Reliabilities using algorithms were excellent for major depression, bipolar
disorder, and schizophrenia but not for schizoaffective disorder, for which disagreement on duration of mood syndromes relative to psychosis reduced reliability 51, 96, 142.

Family Interview for Genetic Studies (FIGS) is a complementary instrument to DIGS for relatives. The aim of the interview is to evaluate the existence of psychopathology in family members for whom no personal interview has been possible to perform. It is useful if the direct information from a patient poses difficulties 142. Thus, in FIGS the symptomatology is not assessed by a personal interview but given by a relative of the persons of interest. Scales for assessment of Positive Symptoms (SAPS) and Negative Symptoms (SANS) were developed for differential assessment of positive and negative symptoms 6-8.

1.3.2 Medical records
In clinical practice the information from psychiatric interviews is summarized in the psychiatric record. Thus the psychiatric record is based on anamnestic and observed information from one or several interviews with the patient, his family, or significant others. In Sweden the record usually contains information describing symptoms and observed behaviour as well as physical examinations, diagnosis and medication. The case notes are usually written directly after or within hours after the meeting between the patient and the care-giver giving an immediate assessment of data, which should not be seriously affected by recall bias.

1.3.3 Register data
The Swedish Psychiatric Inpatient Register (SPIR) started in 1971 and covers all psychiatric inpatient hospitalisations in Sweden since 1973. For each patient the psychiatrist in charge recalls and registers the diagnosis (diagnoses) when the patient leaves the hospital. From 1968 until 1986 this was performed according to ICD-8 219 and 1987-1996 according to ICD-9 220. Since 1997 ICD-
10 has been used. For each hospitalisation one or more ICD diagnosis is recorded in SPIR. Administrative personnel send the individual patient data (identification number, hospital number, dates for admission and discharge, and the diagnosis –(es) number(s)) to a central database.

### 1.4 AETIOLOGY OF SCHIZOPHRENIA

Using the above mentioned criteria the lifetime risk for schizophrenia has been estimated to be about 1% in the general population worldwide. It has since long been observed that schizophrenia runs in families. Family twin and adoption studies in schizophrenia all indicate that genetic factors play a major role. Heritability of schizophrenia has been calculated to be between 66 and 85 percent. Monozygotic twins share 100% of their genes. If one of them has schizophrenia the risk for the other twin to be afflicted with the disorder is about 50%, giving room for non-genetic factors. Dizygotic twins, siblings or children of patients with schizophrenia have a risk of about 7-12% of developing the disorder, and the risk decreases further with increasing genetic distance (Figure 1).

**Fig 1.** Lifetime risk of schizophrenia. Adapted from Gottesman 1991 (used with permission from Oxford University Press)
Thus, this pattern suggests a non-Mendelian mode of transmission and makes simple major gene effects unlikely. Instead a polygenic model, provides the best explanatory fit. Thus, similar to cancer, diabetes, and heart disease, schizophrenia appears to be a complex genetic disorder.

Besides genes, several environmental aetiological risk factors have been proposed. Patients who fall ill with schizophrenia are more likely to have had a history of obstetric complications, such as prematurity, asphyxia and low birth weight. Persons with schizophrenia are more often than expected born in late winter and spring. Prenatal viral infections, famine, paternal loss, urbanicity, ethnic minority membership and cannabis use have also been reported as risk factors for schizophrenia.

One way of describing the aetiology of schizophrenia is a stress-vulnerability continuum in which genetic and environmental risk factors act in an additive or multiplicative manner until a threshold of liability for expression of psychosis is passed. The vulnerability model, assumes that schizophrenia only occurs in a vulnerable individual, with specific thresholds for each individual.

1.5 MOLECULAR GENETIC STUDIES IN SCHIZOPHRENIA

The inherited, or genetic, constitution of an individual is determined by information laid down in his/her Deoxyribo Nucleic Acid (DNA). DNA consists of two strands, each of which is formed by molecular building blocks called nucleotides. Each nucleotide contains one of four so-called bases. Bases from each strand in a DNA molecule interact in a specific way, forming base pairs. The order in which the bases occur in the DNA, the nucleotide sequence, forms the basis of the genetic information. The entire nucleotide sequence of an organism is called the genome. All nucleated cells of an organism contain identical DNA, packaged in chromosomes. In humans there are 23 pairs of chromosomes, including one pair of sex chromosomes. In the last few decades numerous techniques for detailed analyses of DNA have been developed,
which together with especially developed statistical methods, form the basis of molecular genetics.

DNA forms genes, which exert their functions by encoding proteins. There are about 20,000-30,000 human genes. The human genome contains approximately three billion base pairs. Of these only about three percent are found to be parts of genes $^{50,128,205}$.

The genetic make-up influences an individual’s characteristics and susceptibility to disorders. In complex traits, i.e. with non-Mendelian modes of inheritance, such as schizophrenia, different approaches have been developed for identifying genes that increase the risk of the disease. Numerous studies have been carried out aiming at finding chromosomal loci and genes that increase susceptibility for schizophrenia. However, the results obtained have often been inconsistent and difficult to reproduce.

1.5.1 Linkage and association studies

Linkage and association analyses are complementary strategies for mapping parts of the DNA containing disease susceptibility genes $^{169}$. Linkage analyses, and association studies, depend on the presence in the DNA of detectable polymorphic genetic positions (markers), i.e. sites that vary among individuals. With these markers it is possible to systematically scan the complete genome, and test parts of a chromosome and specific candidate genes. There are several types of DNA markers. The most common type of DNA variation is so-called single nucleotide polymorphism (SNP), i.e. a site in the DNA where a single base pair differs among individuals, $^{78,206}$. Another type of frequently used genetic markers is the microsatellite, i.e. a repeat of a simple DNA sequence varying in length between individuals. Microsatellites have proven to be informative and easy to genotype $^{182}$. 
Linkage analysis relies on a genetic process called recombination. Each gene, genetic marker or locus is made up of two alleles, one inherited from each parent. During formation of the sex cells (meiosis), the paternal and maternal chromosomes exchange pieces of genetic material, so that they are recombined. Recombination (or crossing over) seems to occur in a nearly random fashion. Two genetic loci are linked if they are transmitted together from parent to offspring more often than expected under independent inheritance. In general, the closer two loci (positions) are on the same chromosome, the more unlikely it is that a recombination has occurred. Two loci are linked if, during meiosis, recombination occurs between them with a probability of less than 50%.

There are different strategies for performing linkage analysis. The study population is either families in the form of extended pedigrees or affected sib-pairs. A commonly used statistical term in linkage analysis is the “logarithm of the odds ratio” (LOD) score \(^{134,156}\). The LOD score gives a measure of the probability that linkage is occurring.

Linkage analysis can be used to identify regions of the genome that contain genes that predispose to disease. The analysis seeks to find chromosomal positions, loci, within related individuals which differ among affected and unaffected individuals \(^{63}\). Linkage analysis can be applied to both gene disorders with a known mode of inheritance (parametric linkage) and genetically complex diseases (model-free or non-parametric linkage). Linkage analysis is often the first stage in the genetic investigation of a trait, since it can be used to identify broad genomic regions that might contain a disease gene, even when a biologically driven hypothesis is lacking \(^{49}\). Linkage analysis does not identify disease genes, but it can narrow down the parts of the DNA where to search for these genes.
The aim in association studies is to detect alleles of a specific gene that are more or less common in cases than in the general population. Association is a statement about the co-occurrence of alleles and phenotypes. Allele A is associated with disease D if people who have D also have A more (or less) often than would be predicted from the individual frequencies of D and A in the population\textsuperscript{81,182}. In association studies, it is possible to detect genes with minor effects, on small distances in the genome\textsuperscript{36,128}. However, in contrast to linkage studies where a few hundred markers are sufficient to screen the whole genome, a full genome screen by association studies is difficult due to the large number of markers that would have to be included. Therefore, today the researcher has to select and focus on specific candidate genes for this type of study. Selection may be on the grounds of the position of a gene, i.e. it maps on to a chromosomal region implicated by previous linkage studies. Studies can also be performed because of function, i.e. the gene encodes a protein implicated by a pathophysiological hypothesis, e.g. the dopamine hypothesis. If a genuine association appears it may either be a consequence of linkage disequilibrium between the marker investigated and a nearby functional polymorphism, or an effect of the investigated polymorphism itself. Preferably, association studies are performed with polymorphisms known to affect the function of the protein.

Most of the recent findings of interest in schizophrenia have emerged from studies analysing positional candidate genes, although some putative susceptibility gene variants have been suggested on the basis of the function of a protein. Several detailed mapping studies of linked regions have implicated specific genes that increase the susceptibility for schizophrenia. At the time of writing, there are several interesting results.
1.5.2 Molecular genetic findings in schizophrenia

Some seemingly consistent results have been reported. Several research groups have presented evidence of linkage between regions on chr 6 and chr 8 and schizophrenia. Two meta-analyses obtained overlapping, but somewhat different results. One of these studies supported the existence of susceptibility genes on chr 8p, 13q, and 22q. The second study primarily favoured 2q, but also reported evidence for linkage on chr 5q, 3p, 11q, 6p, 1q, 22q, 8p, 20q, and 14p. Thus, the 8p and 22q regions were supported by both meta-analyses, but eight other regions were supported by only one of them. The first generations of linkage studies in schizophrenia ignored the evidence for genetic complexity. Notwithstanding linkage studies in large families with a high prevalence of schizophrenia and related phenotypes have produced positive findings, most of these results have not been replicated. A combination of small genetic effects and inadequate sample sizes may be part of the explanation.

There have been a number of studies showing an association between the dysbindin, or dystrobrevin binding protein 1 gene (DTNBP1) on chr 6p22.3 and schizophrenia. However, there are inconsistencies of the specific risk alleles and haplotypes among studies, suggesting the presence of multiple susceptibility and protective alleles, or that a single susceptibility allele is carried on a remarkable diversity of haplotypes even in closely related populations. The function of dysbindin is largely unknown. It has been proposed that variation in dysbindin might influence the risk of schizophrenia through effects on pre-synaptic glutamate function.

Neuregulin 1 (NRG1), located on chr 8p21-22, is another gene that has been associated with schizophrenia in several reports. This gene is thought to encode about 15 proteins with a diverse range of functions in the brain, including cell-cell signalling, axon guidance, synaptogenesis, glial differentiation, myelination, and neurotransmission. As with regard to the
dysbindin gene, the genetic support for NRG1 is not clear-cut, with association to different markers and haplotypes in different populations. Negative findings have also been reported\textsuperscript{91, 188}.

In the last few years additional reports of association with candidate genes implicated by their chromosomal location, suggested by linkage studies, have attained substantial attention. These additional genes include e.g. regulator of G signalling (RGS4) on chr 1q22\textsuperscript{31, 213}, D-amino-acid oxidase (DAOO) on chr 12q24\textsuperscript{32, 120}, D-amino-acid oxidase activator (DAOA/G72) located on chr 13q22-24\textsuperscript{32, 162}, Disrupted-in-schizophrenia-1 (DISC-1) on 1q42\textsuperscript{19, 85, 148}, and proline dehydrogenase (PRODH) on 22q11\textsuperscript{119, 144, 208, 209}. All these genes have been associated with schizophrenia in some, but not all studies\textsuperscript{147}. Furthermore, as with dysbindin and NRG1, no functional gene variants have been detected and the association patterns are inconsistent among studies.

Catecholamine O-methyl transferase (COMT) has been studied intensively because of its location at chr 22q11\textsuperscript{56, 76, 202, 204}, where small interstitial deletions give rise to the velo-cardio-facial syndrome (VCFS), which in turn is associated with schizophrenia\textsuperscript{138, 154}. In addition, COMT is involved in the dopamine catabolism and as such a functional candidate for schizophrenia. Furthermore, a functional COMT gene polymorphism (Val158Met), altering the enzyme activity of the protein, has been found\textsuperscript{110}. A large Israeli study of over 3000 individuals reported strong evidence for association among three COMT polymorphisms, including the functional one, as well as haplotypes and schizophrenia\textsuperscript{172}. However, most reports have failed to find association and meta-analysis of the functional variant did not report association\textsuperscript{136}.

Alterations in dopamine transmission and dopamine receptors have since long been hypothesized in the pathophysiology of schizophrenia\textsuperscript{26, 198}, mainly because antipsychotic drugs have a high affinity for some dopamine receptors, in particular the dopamine D2 receptor\textsuperscript{113, 166}. Association has been reported
between the dopamine D2 receptor gene \( (DRD2) \) and schizophrenia\(^ {14} \) and two independent meta-analyses support association between a \( DRD2 \) Ser311Cys polymorphism and the disorder\(^ {115} \).

Another of the five dopamine receptor genes, the dopamine D3 receptor gene \( (DRD3) \), has also been intensively analysed in schizophrenia. An SNP changing Serine to Glycine at position 9 in the protein\(^ {111} \) has been analysed in several case-control and family-based association studies. Until recently, all of several meta-analyses performed have reported association with the disorder\(^ {115} \). However, in the most recent and largest meta-analysis so far, including more than 11000 subjects, the association between \( DRD3 \) Ser9Gly homozygosity and schizophrenia just fell short of conventional significance\(^ {95} \).

During the last two decades, the idea that schizophrenia is caused by a disturbance in neurodevelopment has been one of the major hypotheses in biological schizophrenia research\(^ {94,203} \). Brain-derived neurotrophic factor (BDNF) is one of the neurotrophins, regulating survival, differentiation, morphology and synaptic remodelling of neurons\(^ {15} \). BDNF has also been shown to modulate transmitter synthesis, metabolism and release, postsynaptic ion channel fluxes, neuronal activity and long term potentiation\(^ {15} \). BDNF is also involved in the development and survival of dopaminergic and serotonergic neurons\(^ {55,185} \). Thus, the \( BDNF \) gene, located on chr 11p13, is connected to several of possible schizophrenia hypotheses and as such is a candidate for schizophrenia.

The improved molecular genetic and statistical techniques together with publicly available genetic databases and connected resources\(^ {50,157} \) have contributed to the recent advances in psychiatric genetics. However, it is necessary to consider that after 25 years of research in the molecular genetic field, there is still little consistency regarding the importance of specific risk genes for schizophrenia. This inconsistency may have several explanations.
Poor validity and reliability of current diagnostic criteria, heterogeneity of schizophrenia and inappropriate patient materials to disclose the influence of heterogeneity may decrease the likelihood of detecting linkage and association between genetic markers and the disorder.

The present thesis deals with all these questions. However the thesis has its main focus on the recruitment and diagnostic procedures for patients to be included in research in order to further improve prospects for disclosing molecular genetic mechanisms behind schizophrenia.
2 AIMS

The general aim of the present thesis was to search for susceptibility gene loci in schizophrenia.

The specific aims of the studies were:

1. To recruit three different Swedish patient materials with a conceivable diagnosis of schizophrenia.
2. To clinically characterize and certify a research diagnosis of schizophrenia in these patients.
3. To evaluate patient interview data and national register diagnostic data for these patients with schizophrenia.
4. To compare different schizophrenia diagnostic procedures for reliability, validity and practical suitability for genetic studies.
5. To examine and compare the patient materials with regard to linkage and association with molecular markers for genetic loci and some candidate genes in schizophrenia.
3 MATERIAL

3.1 ETHICAL ASPECTS

All the studies were conducted in accordance with the Declaration of Helsinki and were approved by the local/regional Ethics Committees. All subjects participated with written informed consent, given during remission or in a stable phase of their illness.

3.2 SUBJECTS

3.2.1 Patients with schizophrenia

To increase the likelihood of finding risk genes for schizophrenia three different Swedish patient materials with a preliminary diagnosis of schizophrenia were recruited.

1. Sporadic cases with schizophrenia were recruited within the Stockholm County psychiatric service organisation (papers I, V and VI).

2. A large pedigree with a number of cases with schizophrenia in several generations (papers II and III).

3. Swedish sib-pairs with schizophrenia and their first-degree relatives (papers I and IV).

Fig 2, Sweden. The shaded areas indicate the geographical origins of patient materials 1 and 2. Patient material 3 was distributed all over Sweden.
3.2.2 Control subjects
For the sporadic cases with schizophrenia healthy control subjects were selected from staff and students of the participating centers as well as from the general population (papers V and VI). In the large pedigree, healthy first-degree relatives of the patients with schizophrenia were included (papers II and III). In the sib-pair material healthy first-degree relatives of the sibs with schizophrenia were included (paper IV). The reference materials are presented in greater detail in the separate papers.

3.3 RECRUITMENT OF SUBJECTS
3.3.1 Patient material 1
Sporadic cases with a preliminary diagnosis of schizophrenia or a related psychosis were traced through the treating psychiatrists of four psychiatric clinics, specialised for the treatment of psychosis in the County of Stockholm (Fig 2). When the patients had been identified they were asked to participate by their treating nurse or psychiatrist.

3.3.2 Patient material 2
Among the patients treated at the psychiatric clinic of Umeå University Hospital in 1991-92, there were 16 subjects with schizophrenia who lived in the same geographic area, a parish located in the interior of Northern Sweden (Fig 2). This area was isolated, until the early 20th century, when roads and railways were constructed. Therefore possible consanguinity between them was questioned. To analyse this question, the following actions were taken. First there was a search for additional patients living in the parish who had been treated for psychotic illness. This search was performed by using registers and records from the former regional mental hospital, the local psychiatric hospital, and registers of the local primary health care unit. As a second step a genealogical investigation established the familial relationships among the cases. Parish registers and the Ph.D. thesis by Torsten Sjögren were also used for finding additional cases of possible consanguinity. In this way, 40
family-related cases with schizophrenia were identified and 18 small family
trees could be constructed. Using old and computerised church registers it was
possible to connect all these eighteen families twelve generations back to a
single ancestral couple born in the middle of the 17th century. Church registers
were used to connect the small families in this large pedigree (papers II and
III). These registers generally contained information on whether an individual
had been feeble-minded or hospitalised for a psychiatric disorder, thereby
increasing the number of cases identified. By using these procedures
altogether 279 cases treated for a psychiatric disorder were ultimately
identified. During almost ten years of genealogical search, 1991-2000, this
pedigree expanded to include approximately 3,400 individuals. Of those 250
were still alive at the first genetic investigation. These individuals all belonged
to generations nine to twelve of the pedigree (paper II).

3.3.3 Patient material 3
Families from all over Sweden with sib-pairs afflicted with schizophrenia were
traced from the Swedish psychiatric inpatient register (SPIR). Patients who had
an ICD (versions 8, 9 or 10) register diagnosis of schizophrenia or a related
psychosis were identified. By crosschecking the personal identity numbers of
these patients with the Swedish second generation register, the familial
relationship between the subjects could be unravelled. Nation-wide (fig 2)
approximately 1600 sib-pairs could be identified where at least one sibling had
been diagnosed with schizophrenia and where the other sibling had been
treated for a schizophrenic psychosis or psychosis NOS (Ösby, personal
communication). Further details of the subject materials are presented in the
separate papers.

3.3.4 Contact with subjects
When the subject of each patient material had been identified, s/he was first
contacted by letter. One week later the patient was approached by phone and
asked to participate in the projects, by allowing access to his/her medical
records, to participate in an interview and to deliver a blood-sample for molecular genetic studies.
4 METHODS
4.1 CLINICAL ASSESSMENT
The different subject materials were in principle examined with similar methods to determine research diagnostic categories. However, there were some differences in definitions and categories between the various studies. These are described in the following section. The following information sources were used for the diagnostic assessment.

4.1.1 Registers
The Swedish Psychiatric Inpatient Register (SPIR) was used to obtain a register diagnosis for patient materials 1 and 3 (papers I and IV). Local hospital registers and church registers were primarily used to identify cases for patient material 2 (papers II and III).

4.1.2 Medical records
When the patient had given consent, requests for the records were sent to the journal archives of each psychiatric hospital or clinic. In this way, the patients' lifelong psychiatric records were collected. The information from the records was then scrutinized by a research psychiatrist. The record information was summarized in an OPCRIT-protocol (version 3.3 in papers II-III and version 3.4 in papers I and IV)\(^\text{112}\). The ratings were made by the research psychiatrists. All Swedish raters were psychiatrists, trained in the OPCRIT instrument by members of the British team, who initially developed OPCRIT.

4.1.3 Patient interviews
Each patient was interviewed and the aim of the interview was to examine the presence of psychiatric symptoms and to define the type of psychopathology. The interviews were conducted by a psychiatrist (patient materials 1 and 2; papers I-III, V and VI) or a research nurse (patient materials 2 and 3; paper I-IV). The interviews were usually made in the patients’ homes (patient
materials 2 and 3) or at a psychiatric clinic (patient material 1). With a few exceptions the patient interviews were performed face-to-face during non-hospitalisation, i.e. when the patients were in remission or in a stable phase of their illness.

During the course of the present studies there were several changes in the interview procedure. The first patients recruited to materials 1 and 2 were interviewed with an unstructured method. When the international collaboration was initiated in the middle of 1990’s structured interviews were introduced. The research psychiatrist and research nurses were trained to use the different instruments: SCID-I (papers V-VI), DIGS (paper III), SCAN chapters 6, 10, 17, 18 and 19, SAPS and SANS (papers I, IV-VI).

After the interviews, summary vignettes were formulated describing the symptoms and the course of the disorder as presented during the interview. The vignettes emerging from the interviews conducted by the research nurses were discussed and checked by the present investigator. For patients in papers I, IV-VI, a summary protocol was also prepared. The total time spent on the interview and the completion of the interview protocols, spanned between four and eight hours for each patient. There were often considerable travel-distances since the patients were distributed all over Sweden.

4.1.4 Control subject interviews
The control subjects for patient material 1 (papers V and VI) were interviewed using the SCID-I non-patient version. The non-affected relatives of patient material 2 were subjected to unstructured interviews or interviewed with FIGS to check for the existence of psychiatric symptoms/psychopathology, in their healthy or ill relatives, face-to-face and/or over the telephone (papers II and III).
4.2 SELECTION AND CERTIFICATION OF DIAGNOSIS

For all the patients the preliminary diagnosis was re-evaluated to formulate the following research diagnoses of schizophrenia spectrum disorder on the basis of the different information sources. Four categories of research diagnoses were determined. The definition of each category is described in the following section.

4.2.1 Register Diagnosis

Analysis of the register diagnoses was performed in paper I and includes data from patient materials 1 and 3. For each hospitalisation the SPIR diagnoses were recorded according to ICD 8, 9 or 10. Only patients with a SPIR diagnosis of schizophrenia or related psychosis were included in the study. The majority of subjects had experienced several hospital admissions and diagnoses. For the analyses, each patient was given only one register diagnosis. To achieve this goal the following diagnostic hierarchy was used: schizophrenia, schizoaffective disorder, schizophreniform psychosis, psychosis not otherwise specified (N.O.S.), delusional disorder, bipolar disorder, depressive disorder, and any other diagnosis. Thus, if the patients had ever been discharged with a diagnosis of schizophrenia s/he was regarded as having a register diagnosis of schizophrenia. If the patient had never been given a schizophrenia diagnosis, but had been diagnosed with schizoaffective disorder, s/he was regarded to have a schizoaffective register diagnosis and so on following the hierarchy. The ICD diagnoses corresponding to each of the hierarchy diagnoses are summarized in table 2.


<table>
<thead>
<tr>
<th>Diagnostic cluster</th>
<th>ICD-8 diagnosis</th>
<th>ICD-9 diagnosis</th>
<th>ICD-10 diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizoaffective</td>
<td>295.70</td>
<td>295H</td>
<td>F25.0-2, F25.8-9</td>
</tr>
<tr>
<td>Schizophreniform</td>
<td>295.40</td>
<td>295E</td>
<td>F20.8</td>
</tr>
<tr>
<td>Delusional disorder</td>
<td>297.00, 297.10, 297.98</td>
<td>297B, 297C, 297D, 297W, 297X</td>
<td>F22.0, F22.8, F22.9, F24.9</td>
</tr>
<tr>
<td>Depression</td>
<td>296.00</td>
<td>296B</td>
<td>F32.0-3, F32.30-31, F32.8-9, F33.0-4, F33.8-9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other mental disorders</th>
<th>Any other diagnosis</th>
<th>Any other diagnosis</th>
<th>Any other diagnosis</th>
</tr>
</thead>
</table>

### 4.2.2 Standard Research Diagnosis (SRD)

The information from the patients' lifelong psychiatric records as well as the information from the interviews was used in all papers. On this basis, i.e. all the information available, a DSM-IV Standard Research Diagnosis (SRD) was given by the research psychiatrist. In papers II, III and IV the term “diagnosis” represents the SRD as defined in paper I. In papers II and III, this diagnosis
was established independently by the present author as well as the senior psychiatrist. If we disagreed, a consensus diagnosis was made. In papers I and IV, all complicated cases were separately rated by at least two independent raters and a consensus SRD diagnosis was made or the case was excluded if agreement was not achieved. Parts of the patient material I (papers V and VI) included an older patient material, previously given an SRD diagnosis according to DSM-III-R by one of the research psychiatrists involved in the present study 97, as well as more recently investigated patients that were given SRD DSM-IV diagnoses. To compare the different diagnostic systems, 40 patients were evaluated using both DSM systems. All of them received a similar diagnosis irrespective of the system used.

To test the inter-rater reliability, several sessions were performed where the raters independently assessed the same cases for SRD. The inter-rater reliability was calculated in paper I. The level of agreement for SRD between the senior psychiatrist and the present author was excellent (p = 0.90, \( \kappa = 0.84 \)) when independently analysing ten cases. However scrutinization of all this material was a very time-consuming process. Therefore, the senior psychiatrist and a third psychiatrist involved in the diagnostic process of paper I analysed prepared vignettes from records and interview information (n=20), or prepared abstracts from research interviews (n=20). In addition to prepared abstracts they analysed two full cases on which they fully agreed. Altogether, the concordance was good to excellent (p = 0.88, \( \kappa = 0.77 \)).

### 4.2.3 OPCRIT algorithm diagnoses (OPCRIT-R)

After the SRD had been formulated, the OPCRIT algorithm was applied to create additional DSM-IV diagnoses. In paper I, DSM-IV diagnosis was obtained by the OPCRIT algorithm, based exclusively on the record analysis (OPCRIT-R).
4.2.4 OPCRIT algorithm diagnoses (OPCRIT-R+I).
After completing the OPCRIT-R checklist the research psychiatrists also scrutinised the protocols from the interview and completed a second OPCRIT checklist based on the information from both the records and the interview (paper I). From this checklist a DSM-IV diagnosis was also obtained using the algorithm of the OPCRIT computer program (OPCRIT-R+I). In papers II, III and IV the OPCRIT checklists were completed based on the information from both the records and the interview, giving the OPCRIT-R+I diagnoses.

4.3 MOLECULAR GENETIC ANALYSIS
For the genetic analysis venous blood samples were taken. Standard procedures were used for DNA extraction. DNA amplification was performed by polymerase chain reaction (PCR). Polymorphic microsatellite markers were used in the genome scans (papers II-IV). Approximately 370 markers at 10 cM intervals were typed in the genome-wide scans. The genotypes were checked for Mendelian errors. Single nucleotide polymorphisms (SNPs) were used as markers in the case-control association studies and the genotyping was performed by pyrosequencing or cleavage with restriction enzymes (papers V-VI). PCR and molecular genetic analyses were performed according to methods described in detail in the separate papers.

4.4 STATISTICAL ANALYSIS
Paper I
Unadjusted agreement (P) and Cohen’s un-weighted nominal kappa (κ) were calculated to report level of concordance. Sensitivity, specificity, positive predictive power and negative predicted power were also calculated.

Papers II, III, IV
In papers II and III, two-point and three-point linkage analyses were performed using the MLINK and FASTLINK programs, of the LINKAGE package.
The EHPLUS package was used for the haplotype analysis in paper III\textsuperscript{224}. In paper IV, single-point LOD scores were computed using SPLINK\textsuperscript{86}. Multipoint linkage analyses were performed using the MAPMAKER/SIBS package\textsuperscript{109}. The statistical analysis and the different tools are described in detail in the separate papers. Information and access to different kinds of linkage programs may be found on the Internet through the Rockefeller University (http://linkage.rockefeller.edu/soft/list.html).

**Papers V, VI**

The allele and genotype frequencies among cases and controls were compared using contingency and 2 × 2 $\chi^2$-tests\textsuperscript{168}. Odds ratios and confidence intervals, tests for heterogeneity between the different studies and pooling of data were performed according to Woolf\textsuperscript{218}. Power was estimated\textsuperscript{34,57}. To compare ages between cases and controls $t$-test was used. Survival analysis (Kaplan-Meyer) was used to examine possible association between the $DRD2$ and $BDNF$ genotypes and age at onset of illness. Statistical analyses were conducted using the packages StatView and JMP. In paper VI, linkage disequilibrium and haplotype estimation were performed with the programs PHASE v2.1\textsuperscript{181}, Haploview\textsuperscript{17} and R (www.r-project.org).
5 RESULTS

5.1 EVALUATION OF DIFFERENT DIAGNOSTIC PROCEDURES (PAPER I)

In order to compare the different diagnostic procedures for reliability, validity and practical suitability for genetic studies patient records, interview data and national register diagnostic data were examined and evaluated for patients with schizophrenia. In the analysis, data from 143 patients were used. Patients were recruited from sporadic cases (patient material I) and from the sib-pairs (patient material III). There were 83 men and 60 women. The records described inpatient and outpatient treatment periods spanning from seven weeks to 41 years (mean ± S.D. 18.0 ± 9.3 years) and included between 0 and 61 hospitalisations (mean ± S.D. 13.1 ± 13.8). The average number of hospitalization records per patient was 10, but nine patients had never been treated as inpatients. The patients received the following research and register diagnoses according to DSM-IV and ICD 8, 9 or 10, respectively (table 3).
### TABLE 3. DISTRIBUTION OF PATIENTS TO DIAGNOSES IN THE FOUR DIFFERENT DIAGNOSTIC CATEGORIES.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>OPCRIT-R DSM-IV</th>
<th>OPCRIT-R+I DSM-IV</th>
<th>SRD DSM-IV</th>
<th>Reg diag ICD-8, 9, 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizophrenia</td>
<td>91</td>
<td>95</td>
<td>92</td>
<td>93</td>
</tr>
<tr>
<td>Schizoaffective disorder, bipolar(^1)</td>
<td>20</td>
<td>23</td>
<td>27</td>
<td>6(^1)</td>
</tr>
<tr>
<td>Schizoaffective disorder, depressed(^1)</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>-(^1)</td>
</tr>
<tr>
<td>Schizophreniform</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Psychosis N.O.S.</td>
<td>16</td>
<td>11</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Delusional disorder</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Bipolar disorder</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Depression</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Uncertain scz aff bipolar or bipolar(^2)</td>
<td>4</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Uncertain scz aff depressed or depression(^2)</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>143</strong></td>
<td><strong>143</strong></td>
<td><strong>143</strong></td>
<td><strong>134(^3)</strong></td>
</tr>
</tbody>
</table>

\(^1\) Register diagnoses did not differentiate between schizoaffective disorder bipolar or schizoaffective disorder depressed.

\(^2\) The OPCRIT algorithm could not differentiate between schizoaffective disorder bipolar and bipolar disorder as well as schizoaffective disorder depressed and depression for four to five subjects with regard to the OPCRIT-R and OPCRIT-R+I diagnoses. These subjects were excluded in the calculations including OPCRIT-R and OPCRIT-R+I, respectively.

\(^3\) Nine subjects had never been treated as inpatients, and were accordingly not given any register diagnosis.

### Comparison of diagnoses based on records only with diagnoses based on records and interviews

When all the eight different diagnosis categories were analysed, research diagnoses based only on records displayed good to excellent agreements with research diagnoses based on records and interview information (OPCRIT-R vs OPCRIT-R+I \(p=0.93, \kappa=0.89\); OPCRIT-R vs SRD \(p=0.85, \kappa=0.71\)). To further compare the diagnoses before and after the addition of interview information the patients were separated into those with schizophrenia and those with any other diagnoses. The concordance rates were excellent (table 4).
When the subjects were separated into those with schizophrenic psychoses (i.e. schizophrenia, schizoaffective disorder, schizophreniform disorder) and those with any other diagnosis, the concordance rates were fair to excellent (table 5).

**Comparison of register diagnoses and research diagnoses**

When the Swedish register diagnoses were compared with each of the three research diagnoses (OPCRIT-R, OPCRIT-R+I and SRD) the agreement was poor ($\rho$ 0.60 – 0.61, $\kappa$ 0.22 – 0.27). To further compare the register diagnoses with the research diagnoses, the patients were separated into those with schizophrenia and those with any other diagnosis. Concordance rates suggested a poor to fair fit (table 6).
TABLE 6. AGREEMENT STATISTICS COMPARING REGISTER DIAGNOSES WITH RESEARCH DIAGNOSES EVALUATING PATIENTS WITH SCHIZOPHRENIA AND PATIENTS WITH ANY OTHER DIAGNOSIS.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>P</th>
<th>κ</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>P.P.P.</th>
<th>N.P.P.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reg.diag vs OPCRIT-R</td>
<td>0.69</td>
<td>0.29</td>
<td>0.79</td>
<td>0.49</td>
<td>0.75</td>
<td>0.55</td>
</tr>
<tr>
<td>Reg.diag vs OPCRIT-R+I</td>
<td>0.68</td>
<td>0.26</td>
<td>0.78</td>
<td>0.48</td>
<td>0.76</td>
<td>0.50</td>
</tr>
<tr>
<td>Reg.diag vs SRD</td>
<td>0.70</td>
<td>0.32</td>
<td>0.80</td>
<td>0.51</td>
<td>0.75</td>
<td>0.59</td>
</tr>
</tbody>
</table>

P = unadjusted agreement. κ = kappa. P.P.P. = positive predictive power. N.P.P. = negative predicted power.

Approximately 75% of the patients receiving a register diagnosis of schizophrenia also received a research diagnosis of the disorder. However, 41-50% of the patients having a register diagnosis other than schizophrenia, were nevertheless given a research diagnosis of schizophrenia (table 3).

When register diagnoses were compared with the research diagnoses and the patients were separated into those with schizophrenic psychoses (i.e. schizophrenia, schizoaffective disorder, schizophreniform disorder) and those with any other diagnosis, the concordance rates suggested a poor to fair fit (table 7).

TABLE 7. AGREEMENT STATISTICS COMPARING REGISTER DIAGNOSES WITH THE RESEARCH DIAGNOSIS EVALUATING PATIENTS WITH SCHIZOPHRENIC PSYCHOSES AND PATIENTS WITH ANY OTHER DIAGNOSIS

<table>
<thead>
<tr>
<th>Comparison</th>
<th>P</th>
<th>κ</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>P.P.P.</th>
<th>N.P.P.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reg.diag vs OPCRIT-R</td>
<td>0.74</td>
<td>0.22</td>
<td>0.79</td>
<td>0.48</td>
<td>0.89</td>
<td>0.30</td>
</tr>
<tr>
<td>Reg.diag vs OPCRIT-R+I</td>
<td>0.74</td>
<td>0.16</td>
<td>0.78</td>
<td>0.47</td>
<td>0.92</td>
<td>0.24</td>
</tr>
<tr>
<td>Reg.diag vs SRD</td>
<td>0.76</td>
<td>0.22</td>
<td>0.78</td>
<td>0.57</td>
<td>0.94</td>
<td>0.24</td>
</tr>
</tbody>
</table>

P = unadjusted agreement. κ = kappa. P.P.P. = positive predictive power. N.P.P. = negative predicted power.

The subjects, who had a register diagnosis of schizophrenic psychoses, also received a corresponding DSM-IV research diagnosis (schizophrenia, schizoaffective disorder or schizophreniform disorder) in 89-94% of the cases. However, 70-76% of the patients not registered with a diagnosis of
schizophrenic psychosis received a research DSM-IV diagnosis of
schizophrenic psychosis. Thus, there was a large proportion of false negatives,
\[ \text{i.e. subjects who were not registered with schizophrenic psychosis or} \]
\[ \text{schizophrenia diagnosis, but judged by the research psychiatrist to fulfil the} \]
\[ \text{DSM-IV criteria for these disorders.} \]

**Comparison of OPCRIT derived and SRD DSM-IV diagnoses**

In the comparisons between the two different diagnoses of which both were

based on combined record and interview analyses (OPCRIT-R+I versus SRD),

there was an excellent agreement (\( p=0.90, \kappa=0.80 \)). This was also the case

when the subject material was separated into schizophrenia/non-schizophrenia

(table 8).

| TABLE 8. AGREEMENT STATISTICS COMPARING OPCRIT DIAGNOSES WITH SRD
EVALUATING PATIENTS WITH SCHIZOPHRENIA AND PATIENTS WITH ANY
OTHER DIAGNOSIS. |
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparison</td>
<td>( P )</td>
<td>( \kappa )</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>P.P.P.</td>
</tr>
<tr>
<td>OPCRIT-R+I vs SRD</td>
<td>0.95</td>
<td>0.89</td>
<td>0.98</td>
<td>0.89</td>
<td>0.95</td>
</tr>
</tbody>
</table>

\( P \) = unadjusted agreement. \( \kappa \) = kappa. P.P.P. = positive predictive power. N.P.P. = negative predicted power.

When subjects were separated into those with schizophrenic psychoses and

those with any other diagnosis the agreement was good to excellent (table 9).

| TABLE 9. AGREEMENT STATISTICS COMPARING OPCRIT DIAGNOSES WITH SRD
EVALUATING PATIENTS WITH SCHIZOPHRENIC PSYCHOSES AND PATIENTS
WITH ANY OTHER DIAGNOSIS. |
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Comparison</td>
<td>( P )</td>
<td>( \kappa )</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>P.P.P.</td>
</tr>
<tr>
<td>OPCRIT-R+I vs SRD</td>
<td>0.94</td>
<td>0.67</td>
<td>0.96</td>
<td>0.73</td>
<td>0.97</td>
</tr>
</tbody>
</table>

\( P \) = unadjusted agreement. \( \kappa \) = kappa. P.P.P. = positive predictive power. N.P.P. = negative predicted power.

The results indicate that OPCRIT is valid with regard to DSM-IV

schizophrenia and related diagnoses.
5.2 LINKAGE ANALYSIS ON CHROMOSOME 6p (PAPER II)

In the late 1990s several studies analysing pedigrees densely afflicted with schizophrenia reported linkage to this disorder with markers on the short arm of chr 6. Therefore the large pedigree with schizophrenia (patient material 2) was considered suitable for verifying this hypothesis. At the time of the linkage analysis on chr 6p (paper II), the pedigree consisted of about 2000 individuals. To make the analysis manageable within a reasonable period of time, a reduced pedigree was constructed, including only the affected individuals and their closest relatives. Of those 96 individuals, 29 were affected, with the following DSM-IV diagnoses: schizophrenia, schizoaffective disorder depressed type, schizoaffective disorder, bipolar type and psychosis N.O.S. These 29 individuals were defined as having a narrow definition of the disease. Two additional patients who suffered from depression with psychotic symptoms were included for an analysis with a broader definition of psychoses.

Through analysis with 16 markers on the short arm of chr 6 significant linkage to the 6p21-23 region was not found in the total pedigree. In a single branch of the pedigree, linkage was suggested to 6p23 (maximum LOD score 2.6) indicating partial verification of the 6p hypothesis and possible heterogeneity within the family.

5.3 A GENOME WIDE LINKAGE STUDY IN THE LARGE PEDIGREE (PAPER III)

After the first study on 6p, additional related subjects were recruited, and the pedigree increased to approximately 3400 individuals. In this family a genome scan using 371 polymorphic microsatellite markers was performed. To make the analysis manageable, the pedigree was reduced to 520 individuals including the affected individuals and the close relatives, who had given consent to be included in the genome scan. The total number of individuals genotyped was 210. Of these 43 (22 men and 21 women) were affected and received the following DSM-IV diagnoses: schizophrenia (29 cases); schizoaffective disorder, depressed type (6 cases), schizoaffective disorder,
bipolar type (4 cases) and psychosis N.O.S. (4 cases). The mean duration of illness was 27 years. The patients were separated into four different diagnostic categories: 1. Schizophrenia only (Scz). 2. Schizophrenia and schizoaffective depressed type (SAD). 3. Schizophrenia, schizoaffective depressed type, and psychosis N.O.S (Broad 1). 4. All the previously mentioned diagnoses and schizoaffective bipolar type (Broad 2).

LOD score data for the four different diagnostic categories were analysed. A maximum LOD score of 3.45 to the 6q 25.2 region was found when allele frequencies in the Swedish control population were used, compared with a maximum LOD score of 2.59 when the pedigree's allele frequencies were used (Broad 1). When additional markers in the 6q25 region were analysed a maximum LOD score of 6.6 emerged. A 6-cM haplotype segregated with the majority of the affected individuals. Multipoint analysis was performed with the markers in the 6q25 region, and a maximum LOD score of 7.7 was obtained. To evaluate the significance of the genome scan, simulations under the assumption of no linkage were performed. The results showed that a LOD score >2.2 should be regarded as suggestive of linkage, whereas a LOD score >3.7 should be regarded as significant. The results strongly indicate the 6q25 region as a schizophrenia susceptibility locus in this family.

5.4 A GENOME WIDE LINKAGE STUDY IN AFFECTED SIB-PAIRS (PAPER IV)
A multinational genome wide linkage study using 372 polymorphic microsatellite markers was performed in 353 affected sib pairs (ASPs) with schizophrenia. The ASPs were separated into two schizophrenia spectrum diagnostic categories, narrow and broad. For narrow diagnosis, both sibs had been diagnosed with schizophrenia or schizoaffective disorder according to DSM-IV. In addition, the broad category included sib-pairs in which one sibling had a narrow diagnosis, but the other had any non-organic psychotic disorder or schizotypal personality disorder. The Swedish sib-pair material
consisted of 134 (79 narrow) ASPs. In addition 179 (170 narrow) ASPs from Great Britain and 40 (38 narrow) ASPs from the United States were analysed. In the Swedish material, the average number of hospitalization records per patient was 10.

In the Swedish sib-pair material, a maximum LOD score (MLS) of 3.00 in a region located on chr 10q25.3-q26.3 was found. The combined material (narrow diagnosis) showed a LOD score of 3.87 on chr 10q25.3-q26.3. This finding achieved genomewide significance ($P < .05$), on the basis of simulation studies. In two other regions, 17p11.2-q25.1 (MLS = 3.35, broad diagnoses) and 22q11 (MLS = 2.29, narrow diagnoses), evidence for linkage was highly suggestive. Moreover, there was also strong evidence for linkage (genomewide $P < .02$) to 17p11.2-q25.1 in a single British pedigree with schizophrenia (broad diagnosis). In the affected sib-pairs, a region located on the long arm of chromosome 10 appears to carry a susceptibility locus for schizophrenia.

5.5 ASSOCIATION STUDY OF THE DRD2 GENE (PAPER V)

In order to replicate a possible association with the putative functional dopamine D2 receptor gene (DRD2) Ser9Gly polymorphism, assigned to chr 11q22-23, the material of sporadic cases of schizophrenia was examined. There were two sub-samples drawn from the same population. The first consisted of 135 patients (63% men), fulfilling DSM-III-R diagnosis of schizophrenia, and 207 control subjects (63% men). The second sub-sample comprised 38 patients with schizophrenia according to DSM-IV (63% men) and 29 control subjects (62% men). Forty patients were evaluated using both DSM-III-R and DSM-IV and they all received the same main diagnosis according to the two diagnostic systems. The DRD2 311Cys variant was associated with schizophrenia in the total sample ($p < 0.002$). When the sample was divided with regard to gender, the association was apparent in men ($p < 0.002$) but not in women. The results were supported by a meta-analysis of all published case-control studies comprising a total of more than 9000 subjects ($P < 0.001$; OR 1.43, 95% CI
1.16-1.78). The DRD2 Ser311Cys polymorphism appears to add susceptibility to schizophrenia.

5.6 ASSOCIATION STUDY OF THE BDNF GENE (PAPER VI)
A possible association to several brain-derived neurotrophic factor (BDNF) gene variants on chr 11p13 was searched for. There were 187 patients with schizophrenia and 275 controls. No significant robust association was found between four BDNF gene variants and schizophrenia or different subsets of the disorder. Standing alone, the present results cast further doubts about the involvement of the BDNF gene in schizophrenia. However, there were associations between both 270 T-allele and Val66Met homozygosity and schizophrenia in meta-analyses comprising 2328 and 4025 subjects, respectively. Even though the meta-analyses did not remain significant when the most significant studies were excluded, a possible influence of the BDNF gene on schizophrenia cannot be ruled out.
6 DISCUSSION

To ascertain gene related disease susceptibility, estimation of a correct lifetime diagnosis is particularly relevant. Diagnostic reliability and validity are often a problem in psychiatric research. In contrast to other fields in medicine, it is not possible to rely on biological data in making the diagnosis of schizophrenia, since the aetiology and pathogenesis are still unknown. The psychiatrist has to rely exclusively on clinical observations of signs and symptoms from the patient and on information from significant others. Introduction of operational definitions, i.e. explicit diagnostic criteria, has improved the reliability of the schizophrenia diagnosis. DSM-IV is a useful tool for clinical and epidemiological communication, but it would be unwise to think that its criteria select anything that maps to the genotypes. In the field of psychiatric genetics, defining the phenotype in schizophrenia and other mental disorders is a problem as difficult as defining the genotype.

The general aim of the present study was first to select a reliable, valid and cost-effective procedure to certify the lifetime research diagnosis of patients with schizophrenia for genetic studies (Paper I). It was found that the scrutinization of case records contained sufficient information to certify a lifetime research diagnosis of schizophrenia in Swedish patients with mainly chronic forms of psychotic illness. Thus, the patient interview supplied little information to modify the diagnoses obtained from examination of the records. From these results it can be concluded that with the access to the medical records spanning the lifetime psychosis history of the patient, a time-consuming and expensive research interview adds little value for verifying a DSM-IV schizophrenia research diagnosis.

In paper I we also compared the Swedish register diagnoses with research diagnoses obtained using the DSM-IV system. Patients with a register diagnosis of schizophrenia or schizophrenic psychosis (schizophrenia,
schizoaffective disorder or schizophreniform disorder) were evaluated. The subjects, who had a register diagnosis of schizophrenia or schizophrenic psychosis, also received a corresponding DSM-IV research diagnosis of schizophrenia or schizophrenic psychosis in about 75% and 90-95% of the total material, respectively. However, the Swedish inpatient register (SPIR) also contained a large proportion of false negatives, i.e. patients who were not registered with schizophrenia (41-50%) or schizophrenic psychoses (70-76%), but who were judged by the research psychiatrist to fulfil DSM-IV research criteria for these disorders.

The high concordance of register and research diagnoses found in this thesis is of a similar magnitude as previously found in Sweden and other Scandinavian countries in studies assessing the validity of the psychiatric register diagnoses with regard to schizophrenia. Altogether, these and the present results (paper I) indicate that register diagnoses of schizophrenia are based on similar principles in the Scandinavian countries. Thus, for the purpose of large-scale genetic studies of schizophrenic psychoses in Sweden and other Scandinavian countries, the results favour the rationale for enrolment of patients who have a register diagnosis of these disorders, as this should include relatively few false positive cases.

In paper I, excellent concordances were obtained between the OPCRIT-derived DSM-IV diagnoses and the Standard Research Diagnoses (SRD) given subjectively by the research psychiatrist, based on the same sources. Previous studies have presented data indicating that the OPCRIT system is valid with regard to diagnoses according to DSM-III-R and Research Diagnostic Criteria. The data from the present study indicates that OPCRIT derived DSM-IV diagnoses are also valid.
In this study the usefulness of the diagnostic information from the research interview was not assessed separately. This question has been analysed in a separate report \(^{200}\), which indicates that structured interviews performed with Swedish long-term treated psychosis patients during non-hospitalisation are a poor source for the evaluation of psychosis diagnoses, but a good screening instrument for the detection of DSM-III-R schizophrenia.

The patients examined in paper I were not randomly drawn from the population. Rather the sample consisted of patients willing to collaborate in partly demanding biological research. This may have introduced bias in the selection of the patients and limits generalisation from the results obtained.

The patients in paper I were generally interviewed during a stable phase of their illness. It is possible that several symptoms experienced by the patient during the active phases of the illness were missed at the interview, as observed signs are difficult to evaluate by questionnaires also when interviews are performed during hospitalisation \(^{27}\). On the other hand, it is likely that for several other data and symptoms, more reliable answers are obtained during a stable phase than during an active phase when the patients are influenced by their psychotic symptoms. In any case, it cannot be excluded that the timing of the interview may have influenced the degree of discrepancy between the different diagnostic assessments.

The use of the OPCRIT algorithm for the analysis may be both a disadvantage and an advantage. All diagnostic systems are imperfect evaluations of a complex reality. Diagnostic algorithms simplify the reality more than the freely thinking human brain. On the other hand, the OPCRIT algorithm offers the advantage, that the diagnosis is not known, when the items are rated. Therefore, the researcher is less likely to be biased with regard to the main goal, i.e. the lifetime diagnosis.
In paper I, several psychiatrists participated in the diagnostic evaluation. The fact that the same psychiatrists made the diagnostic evaluation, both regarding the case notes and the interview protocols, may be both an advantage and a disadvantage from a methodological standpoint. With regard to the comparison of OPCRIT and SRD diagnoses, it is an advantage that the two different methods of giving a diagnosis were performed by the same person and based on the same sources. The results should thus not be influenced by inter-rater discrepancy. However, the robustness of the methods would have been better evaluated if different raters examined different protocols. Further studies of a randomised patient sample with different raters examining the records and the interview protocols may shed further light upon these methodological issues.

Previous studies\(^{133, 163, 184, 199}\) reported linkage to a susceptibility locus on the short arm of chr 6 in schizophrenia. In order to replicate these findings, the pedigree in paper II was analyzed with 16 markers on this chromosome. Only a single branch of the pedigree suggested linkage to 6p23 (maximum LOD score 2.6), partly supporting the chr 6p hypothesis, and also indicating possible heterogeneity within the family. Later on, this region has been implicated in one of the meta-analysis of schizophrenia linkage studies\(^{118}\). Furthermore, a recent genome scan reported linkage to chromosome 6p24 for a subtype of patients with neurocognitive deficits\(^{80}\). Fine-mapping of this region has repeatedly supported association between markers in the dysbindin gene (\textit{DTNBP1}) and schizophrenia\(^{104, 183, 193, 212}\). Dysbindin has emerged as one of the most promising candidate genes for schizophrenia\(^{211}\).

The present results from the genome scan in the large pedigree (paper III) strongly supports linkage (max LOD 7.7) between chr 6q25 and schizophrenia in this family. Previous and later studies have also reported linkage to the long arm on chr 6 in schizophrenia\(^{24, 114, 116, 124}\) and, more recently, in bipolar disorder\(^{52}\). Linkage disequilibrium analyses of chr 6q13-q26 have suggested trace amine receptor 4 (\textit{TRAR4}) as a candidate gene for schizophrenia\(^{53}\). More recently,
another gene in this region, the quaking homolog, KH domain RNA binding (mouse) \((QKI)\), located on chr 6q26-q27, has been suggested as another possible candidate for schizophrenia. The 12-generation pedigree described in this thesis is currently being further analyzed for variants of this gene. Indirect evidence has shown involvement of \(QKI\) in the myelin regulation of the central nervous system. Myelin plays an important role in the development of a normal brain. Disruption of \(QKI\) might therefore have a role in dysfunctional development and schizophrenia symptoms \(^1,^{122}\).

In the present genome wide linkage study of affected sib-pairs with schizophrenia (paper IV), neither the 6p nor the 6q loci were linked to schizophrenia. However, the sib-pair study indicated that a region located on chromosome 10q25.3-q26.3 appeared to carry a susceptibility locus for schizophrenia. The region identified on chromosome 10 is close to a region that has been implicated in several studies \(^{60, 117, 135, 170}\).

Also, in two other regions (chr 22q11; chr 17p11.2-q25.1) evidence for linkage was highly suggestive in the present sib-pair study (paper IV). Moreover, strong evidence for linkage in the latter region was found in a single British pedigree with schizophrenia. The region on chr 17 was one of the regions displaying evidence for linkage in one of the meta-analysis of schizophrenia genome scans \(^{118}\). Linkage to 22q11 in schizophrenia has received support from a number of studies, and the region is one of only two to have been implicated in both meta-analyses \(^{16, 118}\). The linkage peak at chr 22q also coincides with the region deleted in velo-cardio-facial syndrome (VCFS). VCFS is associated with small interstitial deletions of chromosome 22q11. The phenotype of VCFS is variable, but an increased risk of falling ill with psychosis, especially schizophrenia has been reported \(^{138, 154}\). Recently, two of the genes in the 22q11 region, i.e \(PRODH\) and \(COMT\), have been associated with schizophrenia in some \(^{119, 172, 204}\), but not all studies \(^{207}\).
Alterations in dopamine transmission and dopamine receptors have since long been hypothesized in the pathophysiology of schizophrenia mainly because antipsychotic drugs have a high affinity for some dopamine receptors, in particular the dopamine D2 receptor. Post-mortem and brain imaging studies have also reported dopamine D2 receptor alterations in schizophrenic patients. The dopamine D2 receptor gene (DRD2) is located on chr 11p13. Among the few DRD2 polymorphisms reported to change the amino acid sequence of the dopamine D2 receptor protein, only one has been found in a frequency exceeding one percent in most investigated populations. This polymorphism, giving rise to a replacement of Serine by Cysteine at position 311 of the protein, has been shown to influence the formation of cAMP, thereby indicating a functional effect.

The DRD2 Ser311Cys polymorphism has been investigated repeatedly in schizophrenia, with reports of association in a few studies, whereas the majority of reports did not find association. Paper V of the present thesis was the third individual study to show association between the DRD2 Ser311Cys polymorphism and schizophrenia. The bulk of negative reports prompted us to perform a meta-analysis, displaying a significant association between the 311Cys variant and the disorder (odds ratio 1.4). This result was replicated in an independent meta-analysis. A third study, performed to disclose discrepancies between the two meta-analyses, also favoured association with a similar small risk increase of schizophrenia. The results suggest that the DRD2 gene is one among several of potential susceptibility genes slightly increasing the risk for schizophrenia.

Brain derived neurotrophic factor (BDNF) is one of the neurotrophins, regulating survival, differentiation, morphology and synaptic remodelling of neurons. In the present study (paper VI) we analysed seven different BDNF polymorphisms. Three of these, reported to give rise to changes in the amino acid sequence, were however not informative in the present sample. The
remaining four variants did not show any association with the disorder. However, in meta-analyses (paper VI) there were associations between two of the BDNF polymorphisms and the disorder, although these findings were not robust, as removal of the most significant studies made the meta-analyses no longer significant. This calls for further studies with larger populations.

The varying results obtained in the linkage analyses (papers III and IV) show the importance of the control populations in genetic research. For case-control association studies the selection of the control population is also of ultimate importance. In particular, matching for ethnicity is crucial, because different marker allele frequencies have been demonstrated within different ethnical populations. To circumvent this problem, family-based association studies have been advocated. However, even if the ethnical matching is optimal when parents are used as controls, this sampling process is more likely to give rise to selection bias and insufficiently powered samples, in particular as several of the patient-parent constellations will not be statistically informative. Phenotypic screening of the control population is of greater importance in more common disorders, such as depression or alcohol use disorders, than in disorders such as schizophrenia, comprising one percent or less of the population.

In the last few years several specific genes have been associated with schizophrenia risk in a number of studies around the world. In this thesis, all three materials of patients were clinically characterized and the diagnosis certified in a similar way. Linkage analyses and association studies were performed. From the present and previous results by other investigators the following tentative conclusions can be drawn.

There is no major vulnerability gene that is common to the majority of patients with a research diagnosis of schizophrenia. The results support genetic heterogeneity, and/or the existence of several genes with small effects that may
be linked to the syndromal schizophrenia diagnosis. As for other disorders with complex genetic backgrounds there are several difficulties in schizophrenia molecular genetic studies. To these belong, in addition to technical complexity and the rapidly developing methodology, lack of understanding of functions, regulation and interactions of genes involved \(^{69, 153, 216}\). However, the main difficulties may emanate from problems associated with phenotype definition \(^{81}\).

From available results it may be questioned whether the conventional use of the syndromal concept of schizophrenia as a research diagnosis is useful. A multitude of clinical symptoms, signs, and other endophenotypic manifestations such as cognition disturbances, are found among the disorders included under the concept “schizophrenia”. Genetic association studies of specific signs, symptoms and other pathophysiological features of psychotic patients may be a more meaningful approach. The present results strongly support the Bleulerian view of “Die Gruppe der Schizophrenien” \(^{20}\) with some common features that are, however, etiologically connected to combined actions of different subsets of gene variants.

For future genetic studies it is necessary to find reliable, valid and cost-effective methods of defining subsets of patients with schizophrenia. The patient materials of the present thesis have been assessed with regard to a huge spectrum of specific signs and symptoms. An important question is whether the time-consuming and expensive research interview adds sufficient scientific value for the evaluation of signs and symptoms in schizophrenia compared to the evaluation of medical records? Anyhow, for studies aimed at finding genetic associations, valid estimates of quality and intensity over time of separate disease manifestations as well as larger patient materials are required. The vast symptom variability in the present patient materials would increase the number of variables dramatically with this approach. A future understanding of the genetic mechanisms behind the schizophrenia enigma will
therefore require collaboration between national and international research groups. Biobanks with stored blood samples or DNA together with databases in electronic form containing history, signs, symptoms, and other findings such as in the HUBIN project will be useful for such future studies.
ACKNOWLEDGEMENTS

The work in this thesis was carried out at the Department of Clinical Science, Umeå University, Umeå and the Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden. The studies are based on national and international collaboration and also reflect collaboration between different scientific disciplines – psychiatry, epidemiology and molecular genetics.

I wish to express my sincere gratitude to all collaborators and all the individuals who have participated in the studies that made this multidisciplinary thesis possible. In particular, I wish to mention the following persons:

Professor Rolf Adolfsson, my main supervisor, for introducing me to research and inspiring me to start and continue this project.

Associate Professor Erik Jönsson, my co-supervisor, for your stability and accuracy as well as for your important scientific contributions, incredible memory and generous support. I now know what it really means to scrutinize a paper.

Professor Göran Sedvall, my co-supervisor, for your valuable criticism, for your inspiring talent, scientific excellence, for your support and enthusiasm throughout this study.

Professor Lars Jacobsson, for your support and valuable guidance.

Professor Yasmin Hurd, for your enthusiasm, for believing in my thesis and finding ways to overcome difficulties, for all good and fun lectures ranging from science to cooking!

Peter Nordström, Head of the Psykiatri Centrum Karolinska, for allowing me the opportunity to take time out from clinical duties during the final push toward my thesis.

Nils Lindefors, for support and encouragement as well as for pushing me to complete this thesis even during my clinical duties.

Kaj Forslund, for always being friendly and an expert in clinical schedules.

Gabriella Oxenstierna, you have always given me an extra hand with any problem and given me a lot of joy!

Tove Gunnarsson, for being easy to deal with, for providing interesting discussions with a wonderful edgy humour… and also for introducing me to Ragnhild.

Karin, and all the R51 staff who were friendly despite my absence, or perhaps because of it.

Håkan Hall for ambitious organisational work. Diana Radu, for your “sisu” not only with the computer. Urban Hansson, for always being on ”computer”-call.

The Psychiatry R5:00 research corridor for all the “fika”.

Urban Ösby, for introducing me to the sib-pair-study. Per-Olof Nylander for introducing me to psychiatric genetics. Lena Brandt and Andreas Ekholm, for invaluable statistical support.

Carina Schmidt, for introducing me to Stockholm and for giving me support. Marita Signarsson for helping me with administration. Margaretha Lindh, for your enthusiasm, excellent assistance and always making me feel safe during this critical

National collaborators, Agneta Gunnar, Bodil Edman-Ahlbom, Anna Sillén, Lars Terenius, Elena Jazin, Karolina Åberg and last but not least Eva Lindholm, who helped improve my understanding of molecular genetics.

International collaborators, Mike Owen and Nigel Williams, for providing an educational environment for molecular genetics during my visit to your lab.

For all my good friends who I may have seemed to abandon while writing this thesis. Thanks for understanding… Are you still there?

Ola, for all surprises and laughter as well as for your support and for always giving me new things to look forward to!

My family

Mattias, my son, for love and teaching me what is most important in life.

Frida, my daughter for love, fight, humour and your willingness to “have dinner over the phone with me”.

Elisabeth, my sister, for helping me to organise my life both inside and out, for sharing joys and sorrows, and for having a lot of fun together.

Nils-Olof, my brother, for always being there with a positive attitude and for all your technical assistance.

Torbjörn, my brother-in-law, for crisis management…at the last moment.

My parents, Inga and Herbert, for all your care, support and love, and for always seeing opportunities instead of obstacles.

*These studies were supported by the National Institute of Mental Health, NIMH, the Tercentenary Fund of the Bank of Sweden, the Wallenberg Foundation, the Sunrise Trust, the Euraona Ltd, Axys Pharmaceuticals and Parke-Davis, the Swedish Medical Research Council, the Beijer Foundation, Torsten and Ragnar Söderberg Foundation, Söderström-Königska Foundation, the Lundbeck Foundation, Organon Stiftelsen, Stiftelsen Bror Gadelius Minnesfond and the HUBIN-project.*
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