KARI HÄNNINEN

# Selected Genetic Polymorphisms of Epidermal Growth Factor, Dopamine-2 Receptor, Neuregulin-1 and Inflammatory Cytokines in Schizophrenia

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ACADEMIC DISSERTATION
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on April 17th, 2014, at 12 o'clock.

UNIVERSITY OF TAMPERE

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*To my family*

*Contents*

# ABSTRACT

**Background.** The neurodevelopmental hypothesis of schizophrenia suggests that the disease is the behavioural outcome of aberration in neurodevelopmental processes that begins long before the onset of clinical symptoms and is caused by a combination of environmental and genetic factors. Genetic factors are suggested to be in a major role in the etiology of schizophrenia with a heritability of 80%. According to the dopaminergic hypothesis of schizophrenia there is a dopaminergic imbalance in a schizophrenic brain with excessive dopaminergic transmission in mesolimbic projections and defective dopaminergic transmission in mesocortical connections.

**Aim.** The aim of this thesis was to investigate pathogenetic mechanisms in schizophrenia on the basis of the neurodevelopmental and dopamine theory by studying the association of single nucleotide polymorphisms (SNP) in seven candidate genes and the risk of schizophrenia [interleukin-1 alpha (IL-1A) rs1800587, interleukin-1 beta (IL-1B) rs16944, interleukin-1 receptor antagonist (IL-1RN) rs380092, epidermal growth factor (EGF) rs4444903, tumour necrosis factor-alpha (TNF-α) rs1800629, dopamine D2 receptor (DRD2) rs6277 and neuregulin-1 (NRG-1) rs35753505] and the association of five candidate genes and the age of onset of schizophrenia (IL-1B, EGF, TNF-α, DRD2, NRG-1). We also tested the interaction between the IL-1B and NRG-1 genes in schizophrenia.

**Subjects and methods.** The sample consisted of 50 (I), 149 (II and III), 188 (IV), and 113 (V) Finnish patients with a DSM-IV diagnosis of schizophrenia. In the control group there were 400 (I), 393 (II, V), 94 (III), and 384 (IV) healthy blood donors. DNA was isolated from whole blood. Genotypes were determined by using polymerase chain reaction (PCR)-based methods and by employing the 5' nuclease assay.

**Results.** We found that a certain allele combination of the IL-1 gene complex (IL-1A-889 allele 2, IL-1B-511 allele 1, IL-1RN (VNTR) allele 1) was associated with schizophrenia (P=0.027, corrected for multiple testing). The number of IL-1A (-889) allele 2, IL-1B (-511) allele 1 and IL-1RN allele 1 homozygotes was significantly higher in schizophrenic patients than in controls (P=0.0006, corrected for multiple testing). The DRD2 C957T polymorphism (rs6277) was associated with the risk of schizophrenia (pointwise P=0.050). In male patients, the TNF-α –G308A polymorphism (rs1800629) was associated with schizophrenia (pointwise P=0.025, OR 2.00, 95% CI 1.08–3.70), whereas in female patients or in the whole study group no association was seen. The interaction between the IL-1B-511 (rs16944) and neuregulin-1 SNP 221533 (rs35753505) polymorphisms was associated with the risk of schizophrenia (pointwise P=0.008). The IL-1B -511 polymorphism was associated with the age of onset of schizophrenia (pointwise P=0.012). The EGF A61G polymorphism (rs4444903) was associated with the age of onset of schizophrenia in the entire patient population and in male patients, but not in female patients (pointwise P=0.008, P=0.005 and P=0.46, respectively).

**Conclusions.** The results of this study show evidence that polymorphisms of the genes involved in the neurodevelopment of the central nervous system both associate with the risk of schizophrenia and modify the disease by affecting the age of onset. This gives support to the neurodevelopmental hypothesis of schizophrenia. In addition, our results support the dopaminergic hypothesis of schizophrenia. Concerning some genes, there are clear differences between males and females in the risk of schizophrenia and the age of onset of the disease, which refers to other factors (hormonal, for example) potentially interacting with these genes. The effect of one gene was seen to be only modest, which is well in line with other studies. However, our findings suggest preliminarily that interactions between neurodevelopmental genes might have a substantially more prominent effect on the risk of schizophrenia than a single gene has. Future studies are needed to replicate these results and to determine the biological mechanisms that connect the genetic variation of these genes to the genesis of psychosis.

# LIST OF ORIGINAL PUBLICATIONS

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

I Katila H, Hänninen K, Hurme M. Polymorphisms of the interleukin-1 gene complex in schizophrenia. Mol Psychiatry 1999;4:179-181.

II Hänninen K, Katila H, Rontu R, Mattila KM, Hurme M, Lehtimäki T. Tumor necrosis factor-alpha –G308A polymorphism in schizophrenia in a Finnish population. Neurosci Lett 2005;385:76-81.

III Hänninen K, Katila H, Anttila S, Rontu R, Maaskola J, Hurme M, Lehtimäki T. Epidermal growth factor a61g polymorphism is associated with the age of onset of schizophrenia in male patients. J Psych Res 2007;41:8-14.

IV Hänninen K, Katila H, Kampman O, Anttila S, Illi A, Rontu R, Mattila KM, Hietala J, Hurme M, Leinonen E, Lehtimäki T. Association between the C957T polymorphism of the dopamine D2 receptor gene and schizophrenia. Neurosci Lett 2006;407:195-198.

V Hänninen K, Katila H, Saarela M, Rontu R, Mattila KM, Fan M, Hurme M, Lehtimäki T. Interleukin-1 beta gene polymorphism and its interactions with neuregulin-1 gene polymorphism are associated with schizophrenia. Eur Arch Psychiatry Clin Neurosci 2008;258:10-15.

# ABBREVIATIONS AND DEFINITIONS

| | |
|---|---|
| ASH2L | Absent, small, or homeotic-like 2 |
| ADHD | Attention-deficit hyperactivity disorder |
| ANK 3 | Ankyrin 3 |
| ANOVA | Analysis of variance |
| APA | American Psychiatric Association |
| BOLD | Blood oxygenation level-dependent |
| Bp | Base pair |
| BRP44 | Brain protein 44 |
| CCDC68 | Coiled-coil domain containing 68 |
| CI | Confidence interval |
| CNS | Central nervous system |
| CNV | Copy number variation |
| COMT | Catechol-O-methyltransferase |
| CpG | Cytosine-guanine dinucleotide |
| CT | Computed tomography |
| Cys | Cysteine |
| DA | Dopamine |
| D1 | Dopamine-1 |
| D2 | Dopamine-2 |
| DAAO | D-amino acid oxidase |
| DAO | D-amino acid oxidase activator |
| DDHD2 | DDHD domain containing 2 |
| Del | Deletion |
| DGCR8 | DiGeorge syndrome critical region gene 8 |
| DISC1 | Disrupted in schizophrenia 1 |
| DLPFC | Dorsolateral prefrontal cortex |
| DNA | Deoxyribonucleic acid |
| DNMT | DNA methyltransferase |
| DRD2 | Dopamine-2 receptor |
| DRD4 | Dopamine-4 receptor |
| DSM-III | Diagnostic and Statistical Manual of Mental Disorders, Edition 3 |
| DSM-III-R | Diagnostic and Statistical Manual of Mental Disorders, Edition 3, Revised edition |
| DSM-IV | Diagnostic and Statistical Manual of Mental Disorders, Edition 4 |
| DSM-V | Diagnostic and Statistical Manual of Mental Disorders, Edition 5 |
| DTNBP1 | Dystrobrevin binding protein-1 |

| | |
|---|---|
| DZ | Dizygotic |
| EDTA | Ethylenediaminetetracetic acid |
| EGF | Epidermal growth factor |
| EGFR | Epidermal growth factor receptor |
| FGF | Fibroblast growth factor |
| FGFR1 | Fibroblast growth factor receptor 1 |
| fMRI | Functional magnetic resonance imaging |
| GABA | Gamma-aminobutyric acid |
| GAD67 | Glutamic acid decarboxylase 67 |
| GAP | GTPase-activating protein |
| GPCR | G-protein-coupled receptor |
| GTPase | Guanine triphosphatase |
| GWAS | Genome-wide association study |
| HB-EGF | Heparin-binding epidermal growth factor-like growth factor |
| HDAC1 | Histone deacetylase 1 |
| HHAT | Hedgehog acyltransferase |
| HLA | Human Leukocyte Antigen |
| 5-HTTLPR | Serotonin transporter promoter gene region |
| ICD-10 | International Classification of Disease, tenth edition |
| IQ | Intelligence Quotient |
| Ins | Insertion |
| LD | Linkage disequilibrium |
| LETM2 | Leucine zipper-EF-hand containing transmembrane protein 2 |
| LSM1 | U6 snRNA-associated Sm-like protein |
| LTD | Long-term depression |
| LTP | Long-term potentiation |
| mB | Megabase |
| MGB | Minor groove binder |
| MIR137 | MicroRNA 137 |
| MMN | Mismatch negativity |
| MPZL1 | Myelin protein zero-like 1 |
| MRI | Magnetic resonance imaging |
| mRNA | Messenger ribonucleic acid |
| MZ | Monozygotic |
| Neuropil | Complex net of interwoven cytoplasmic processes of nerve cells (dendrites and axons) and of neuroglial cells that forms the bulk of the grey matter in the central nervous system |
| NKAPL | NFKB activating protein-like |
| NMDA | N-methyl-D-aspartate |
| NRG-1 | Neuregulin-1 |
| NRXN1 | Neurexin-1 gene |
| OAT | Ornithine aminotransferase |
| OPCML | Opioid binding protein/cell-adhesion molecule-like |

| | |
|---|---|
| OR | Odds ratio |
| PAGE | Polyacrylamide gel electrophoresis |
| PBL | Peripheral blood lymphocyte |
| PCR | Polymerase chain reaction |
| PDE4B | Phosphodiesterase 4B, cAMP-specific |
| PGBD1 | PiggyBac transposable element-derived 1 |
| PPAPDC1B | Phosphatidic acid phosphatase type 2 domain containing 1B |
| PRODH | Proline dehydrogenase |
| PRSS16 | rotease, serine, 16 (thymus) |
| RA | Receptor antagonist |
| RELN | Reelin |
| RFLP | Restriction fragment length polymorphism |
| RGS-4 | Regulator of G-protein signalling-4 |
| RNA | Ribonucleic acid |
| RT-PCR | Real-time Polymerase chain reaction |
| SAM | S-adenosylmethionine |
| SCID-I/CV | Structured Clinical Interview for DSM-IV Axis I Disorders Clinician Version |
| SD | Standard deviation |
| Ser | Serine |
| SHH | Sonic hedgehog |
| SNP | Single nucleotide polymorphism |
| SPSS | Statistical Package for the Social Sciences |
| SULT6B1 | Sulfotransferase family, cytosolic, 6B, member 1 |
| TCF4 | Transcription factor 4 |
| TGF-α | Transforming growth factor alpha |
| TNF-α | Tumour necrosis factor alpha |
| TPH | Tryptophan hydroxylase |
| TSPAN18 | Tetraspanin 18 |
| VCFS | Velocardiofacial syndrome |
| VIPR2 | Vasoactive intestinal peptide gene 2 |
| VNTR | Variable number of tandem repeats |
| VRK2 | Vaccinia-related kinase 2 |
| WAIS-III | Wechsler Adult Intelligence Test - III |
| WHO | World Health Organization |
| WSPT | Word serial position test |
| WTCCC | Wellcome Trust Case Control Consortium |
| ZDHHC8 | Zinc finger, DHHC-type containing 8 |
| ZKSCAN4 | Zinc finger with KRAB and SCAN domains 4 |
| ZNF | Zinc finger protein |

# 1. INTRODUCTION

Schizophrenia is a chronic, severe brain disorder with an incompletely defined etiology and pathophysiology. Family, twin, and adoption studies have shown a significant role for genetic factors in schizophrenia with heritability of 80% (Cardno and Gottesman 2000). However, because schizophrenia does not seem to follow Mendelian patterns of inheritance, it is suggested that genes interact with environmental factors in the etiology of schizophrenia.

In recent years, the neurodevelopmental model has been the leading explanatory theory in schizophrenia. This model suggests that neurodevelopmental processes are disturbed long before the onset of clinical symptoms, most likely already in utero or in childhood. In schizophrenia, computed tomography (CT) and magnetic resonance imaging (MRI) studies have shown signs of ventricular enlargement and loss of cortical volume which are apparent by the time the disease presents, and they have been considered essentially non-progressive thereafter (Marsh et al. 1994). However, recent studies have suggested that these changes in schizophrenic brains could be progressive in nature (van Haren et al. 2008, Tomelleri et al. 2009, Kempton et al. 2010). Histological studies have detected cortical cytoarchitectural alterations with misplaced, mis-sized and disorganised neurons in schizophrenic brains (Harrison 1997).

The dopaminergic theory of schizophrenia suggests that aberrations in the dopaminergic neurotransmission of the CNS lie behind the positive and negative symptoms of schizophrenia (Seeman and Lee 1975, Creese et al. 1976, Goldman-Rakic et al. 2000). According to this hypothesis, subcortical mesolimbic dopamine

projections are hyperactive, resulting in hyperstimulation of dopamine-2 (D2) receptors and positive symptoms, while mesocortical dopamine projections to the prefrontal cortex are hypoactive, leading to hypostimulation of dopamine-1 (D1) receptors, negative symptoms and cognitive impairment (Davis et al. 1991).

In this thesis, we studied the genetic basis of schizophrenia, on the basis of the neurodevelopmental and dopamine theory, by investigating the possible association between genetic polymorphisms of certain genes affecting the development of the CNS (IL-1A, IL-1B, IL-1RN, TNF-α, EGF, NRG-1) and the dopaminergic neurotransmission (DRD2) and the occurrence of schizophrenia and the age of onset of the disease.

# 2. REVIEW OF THE LITERATURE

## 2.1 Epidemiology of schizophrenia

Schizophrenia is a severe and chronic neuropsychiatric disorder with a lifetime prevalence of about one percent throughout the world. A comprehensive Finnish general population survey showed a lifetime prevalence of 0.87% for schizophrenia with no sex difference in the prevalence of schizophrenia (Perälä et al. 2007). In a meta-analysis by McGrath et al. (2004) the median incidence rate was 15.2 per 100,000 with the 10% and 90% quintiles between 7.7 and 43.0 per 100,000. There is evidence that the incidence could be slightly higher in males than in females (McGrath et al. 2004). Among men the median age of onset is in the early to mid-20s, whereas the onset is 3 to 5 years later in women. There also seems to be a smaller peak in women later — near menopause at ages 40 to 45 years (Hafner and an der Heiden 1997).

Epidemiological studies have shown that being born in an urban versus rural region may be a risk factor for schizophrenia (Marcelis et al. 1998, Haukka et al. 2001). In addition, Haukka et al. (2001) have demonstrated significant regional clustering of schizophrenia in Finland, indicating that either genetic or environmental risk factors that operate early in life have enriched there. Migration has been demonstrated to be a risk factor for schizophrenia by several studies (Zolkowska et al. 2001, Cantor-Graae et al. 2003), but all studies have not found this association (McGrath et al. 2001). The seasonal birth pattern of schizophrenia patients differs from that of the general population. In the Northern Hemisphere, subjects who later develop schizophrenia are more often born in the months from January to April (Pallast et al. 1994, Chen et al. 1996, Torrey et al. 1996) and in the Southern

Hemisphere from July to September (McGrath et al. 1995, Berk et al. 1996). The winter-spring excess has been 5–8% in different studies (see a review by Torrey et al. 1997).

## 2.2 Clinical features of schizophrenia

Schizophrenia is a clinical diagnosis, characterised by an admixture of positive, negative, mood and motor symptoms whose severity varies across patients and through the course of the illness. Positive symptoms involve impaired reality testing and include delusions, hallucinations, and other reality distortions. Most frequent are persecutory delusions or delusions of reference, but delusions of control, thought insertion, withdrawal and broadcasting also exist. Hallucinations can occur in any of the five sensory modalities, but auditory hallucinations are the most common. Among auditory hallucinations, threatening or accusatory voices speaking to the person are most common. In addition, voices can converse among themselves or comment on the patient. Typical of schizophrenia is bizarre content and mood incongruence of the psychotic symptoms. Positive symptoms usually first begin in adolescence or early adulthood, but are often preceded by varying degrees of negative or cognitive symptoms.

Negative symptoms involve a blunting or loss of a range of affective functions. These include impairments in affective experience and expression, abulia (loss of motivation), alogia (poverty of speech), anhedonia (inability to experience pleasure), avolition (lack of initiative), apathy (loss of interest), and reduced social drive (Crow 1980). The pathophysiology of negative symptoms is incompletely understood (Keshavan et al. 2008) and they remain a relatively treatment-refractory and debilitating component of schizophrenic pathology (Stahl and Buckley 2007).

Formal thought disorder in schizophrenia refers to fragmentation of the logical, progressive, and goal-directed nature of normal thought processes and can range in severity from the relatively mild circumstantiality and tangentiality to the more severe incoherence and even word salad. It can include both derailment and neologisms and poverty of thought content. Disorganisation of behaviour often co-occurs with formal thought disorder.

Disturbances of the extent and nature of psychomotor activity are common in schizophrenia. Slowing of psychomotor activity is frequently manifested in schizophrenic patients (Morrens et al. 2007). Excessive motor activity, often apparently purposeless, is more frequently associated with exacerbations of positive symptoms.

Abnormalities of psychomotor activities can vary greatly, from simple isolated movements of posturing, stereotypies and mannerisms to more complex patterns of motions, as seen in various catatonic states. The catatonic syndrome can occur in the context of stupor or excitement, and is characterised by echopraxia, echolalia, waxy flexibility, and extreme negativism. In recent decades, catatonic symptoms have become infrequent as presentations of schizophrenia (Stompe et al. 2002) and are more often observed in mood disorders (Taylor and Fink 2003). These findings suggest that catatonia may not be part of the core psychopathology of schizophrenia (Taylor and Fink 2003, Tandon and Maj 2008).

Impairments in affective expression and experience have long been considered to be cardinal features of schizophrenia and often precede the onset of the psychosis by several years (Hafner and an der Heiden 1999). Depression is common in schizophrenia and may be part of the prodrome, the florid phase, follow an acute psychosis (postpsychotic depression), or occur between psychotic exacerbations.

## 2.3 Cognitive impairment in schizophrenia

In addition to the diverse symptoms that form the clinical definition of the illness, the disorder is, to a variable degree, accompanied by a cognitive impairment. There is evidence that compromised cognitive functioning is a core feature of schizophrenia and not an artefact of other symptoms, treatment for the illness, or aspects of the course of the illness (Kremen et al. 2000). It has further been suggested that the well-known diversity of schizophrenia in terms of functional outcome and recovery from the illness is best characterised by cognitive deficits, not by the classical symptoms (Green et al. 2000). Concerning the general intellectual ability, as measured by the intelligence quotient (IQ) and neuropsychological composite scores, schizophrenia patients show in meta-analytic studies a substantial and very severe generalised impairment (a summary of the results from meta-analytic studies has recently been published by Reichenberg [2010]). According to this review, the effect size of the average impairment of general intellectual ability (IQ) in schizophrenia is over 1 (Reichenberg 2010). Also concerning specific cognitive abilities, meta-analytic studies have revealed remarkable impairments in schizophrenia patients. Especially attention, declarative memory, executive functions, processing speed, and working memory show severe impairment (effect size over 0.8), whereas motor speed, language and perception were moderately impaired (effect size 0.5–0.8) (Reichenberg 2010).

## 2.4 Diagnostics of schizophrenia

The nature of schizophrenia is complex and it has been challenging to develop a reliable definition for the disease. The World Health Association (WHO) and the American Psychiatric Association (APA) have produced criterion-based classifications for diagnosing schizophrenia that have been shown to improve reliability substantially at both diagnostic and symptom level. The most used versions of these classifications are the International Classification of Disease (ICD-10) (World Health Organization 1994) and the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (American Psychiatric Association 1994) (Table 1).

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

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

1. Delusions
2. Hallucinations
3. Disorganized speech (e.g., frequent derailment or incoherence)
4. Grossly disorganized or catatonic behavior
5. Negative symptoms (i.e., affective flattening, alogia, or avolition)

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

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

C. Duration: Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully

treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).

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

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

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

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In both systems, the symptoms must be somewhat persistent or chronic, and they cannot be explicable as secondary to some other recognisable medical condition. Both also use exclusionary criteria to rule out transient psychotic syndromes or psychotic disorders that occur in the context of mood disorders. They also exclude psychotic syndromes that are secondary to general medical illnesses or the effects of various psychosis-inducing substances. The main differences between the DSM and ICD systems are that DSM requires duration of six months compared to only one month for ICD, and deterioration in functioning, which is not presumed by ICD. In both classifications, schizophrenia has been divided into subtypes. In the ICD-10 system there are nine subtypes: paranoid schizophrenia, hebephrenic schizophrenia, catatonic schizophrenia, undifferentiated schizophrenia, post-schizophrenic depression, residual schizophrenia, simple schizophrenia, other schizophrenia, and schizophrenia, unspecified. DSM-IV divides schizophrenia into five subtypes: paranoid schizophrenia, disorganized schizophrenia, catatonic schizophrenia, undifferentiated

schizophrenia, and residual schizophrenia. ICD-10 is the official system for clinical diagnoses in Europe, whereas DSM-IV is used in the United States. The DSM-IV criteria are used for most research studies (Harrison 1999). Structured interviews have been developed for the DSM-IV system to enhance the reliability of schizophrenia diagnosis (First et al. 1997). The diagnosis of schizophrenia is considered reliable (Andreasen 1995, Harrison 1999).

The new DSM-5 classification, published in May 2013, contains several changes concerning the diagnosis of schizophrenia compared to DSM-IV (www.psychiatry.org/dsm5). Firstly, in DSM-5 two Criterion A symptoms are always required for the diagnosis of schizophrenia and at least one of them has to belong to these three core "positive symptoms": delusions, hallucinations, and disorganised speech. Secondly, the DSM-IV subtypes of schizophrenia have been eliminated from DSM-5 because of their limited diagnostic stability, low reliability, and poor validity. A new feature in DSM-5 is a dimensional approach for assessing the severity of the symptoms of schizophrenia. The severity of these dimensions — positive, negative, mood, disorganisation, motor, and cognitive — can vary among patients and also within patients at different stages of their illness. By measuring the relative severity of these symptom dimensions throughout the illness course, clinicians can get valuable information about the nature of a patient's schizophrenic illness and the specific effect of treatment on different aspects of his or her illness.

## 2.5 Age of onset of schizophrenia

Schizophrenia often begins in young adulthood and has severe negative consequences on education, social relationships and employment. There is increasing evidence supporting a distinct difference between early onset and adult onset of the disease (Tuulio-Henriksson et al. 2004, Kester et al. 2006, White et al. 2006). An earlier onset has been shown to correspond linearly with the severity of the course of illness and it also appears to have prognostic value (Rabinowitz et al. 2006). Studies have shown that an earlier onset, as compared to a later age of onset, is associated with poorer educational adjustment (Lay et al. 2000), poorer work history (Jeste et al. 1995), poorer response to antipsychotic medication (Nimgaonkar et al. 1988, Meltzer et al. 1997, Hollis 2000), less extent of improvement (Loebel et al. 1992), more hospital-based care (Rabinowitz et al. 2006), and more severe cognitive deficits (Rajji et al. 2009).

The age of onset of schizophrenia is traditionally defined as the time at which positive psychotic symptoms or disorganisation first appear (Meltzer et al. 1997). This is true, although negative symptoms and cognitive dysfunction are likely to predate the first appearance of psychosis (Bilder et al. 1992, Haas et al. 1992). Prodromal symptoms, like signs of behavioural dysfunction and subclinical psychotic symptoms, often precede the onset of clinical symptoms (Lieberman et al. 2001b). Another way to define the age of onset of schizophrenia is the age at the beginning of the first hospitalisation for schizophrenia (Suvisaari et al. 1998).

Numerous studies have shown that age of onset in schizophrenia is a function of gender. Meltzer et al. (1997) analysed nine studies of age of onset in relation to gender, which were performed in years 1984–1994 using the DSM-III and DSM-III-R diagnostic criteria, and found that females have a later age of onset than males, with differences ranging from 2.9 to 5.4 years. This finding agrees with those from earlier studies of gender and age of onset (Meltzer et al. 1997). The gender-bound difference in age of onset in schizophrenia is suggested to be due to the protective antidopaminergic effect of estrogens in women (Rao and Kolsch 2003). This estrogen effect could also explain the smaller peak in the incidence of schizophrenia observed in women aged 45 to 54 years (Häfner et al. 1993). There are, however, some reports which show no gender difference in age of onset (Ganguli and Brar 1992, Suvisaari et al. 1998), or the gender difference in age of onset is seen only in a specific subpopulation of patients (paranoid patients with age of onset over 30 years) (Salokangas et al. 2003).

There has also been recent evidence that genetic factors significantly contribute to the age of onset of psychotic symptoms in patients with schizophrenia (Hare et al. 2010). In their study, Hare et al. (2010) found that the heritability of age of onset of psychosis was 0.33. On the other hand, this also indicates that there is a large environmental contribution to the age of onset of schizophrenia. One potential environmental factor affecting the age of onset of psychotic symptoms is substance use. A recent study has suggested that pre-onset substance use, especially cannabis use, may have a significant effect in hastening the onset of psychotic symptoms in first-episode patients (Compton et al. 2009).

## 2.6 Environmental risk factors in schizophrenia

The genetic basis of schizophrenia is well established, but those candidate genes that have been identified increase the risk of illness by only two- to threefold (Harrison and Weinberger 2005). Thus, it is probable that multiple susceptibility genes interact among themselves, and probably with environmental factors to manifest as schizophrenia (Weiser et al. 2005). In fact, several environmental factors have been found to be associated with increased risk of schizophrenia.

There is evidence that being exposed to malnutrition in prenatal life increased rates of schizophrenia during the Dutch Hunger Winter of 1944–45 (Susser et al. 1996). Also, prenatal infections have been found to be risk factors for schizophrenia: influenza (Mednick et al. 1988, Cannon et al. 1996), rubella (Brown et al. 2000), poliovirus (Suvisaari et al. 1999), and herpes simplex virus (Buka et al. 2001). Diverse psychological stressors, such as death of spouse (Huttunen and Niskanen 1978), and catastrophic events like war (Van Os and Selten 1998) and nuclear explosion (Imamura et al. 1999) have been shown to increase risk of schizophrenia in offspring in mid-gestation.

There are numerous studies indicating that many obstetric complications appear to increase the risk of schizophrenia (Geddes and Lawrie 1995, Cannon 1997). Cannon et al. (2002) performed a meta-analysis of published population-based studies of obstetric complications as risk factors for schizophrenia and found that the effects of individual complications appeared to group into three main categories: complications of pregnancy, abnormal fetal growth and complications of delivery.

Seasonality of birth is one of the most studied environmental factors in schizophrenia. Most of the studies have observed an excess of winter-early spring births and/or a decrease of late spring-summer births in the disease (Torrey et al. 1997, Tochigi et al. 2004). The effect of seasonality is minor. The risk of schizophrenia for those born during winter-spring was around 5–10% higher compared with the expected number of births during the season, in most of the previous studies (Torrey et al. 1997, Mortensen et al. 1999). Reasons for the seasonality of birth in schizophrenia have been widely studied and possible factors have been identified, among others: meteorologic factors (especially ambient temperature), infections, nutrition, exposure to heavy metals, paternal factors, maternal hormones, and factors involved in infant development after birth (Tochigi et al. 2004). Thus far, however, it has not been proven whether these factors contribute to the development of the seasonality of schizophrenia births (Tochigi et al. 2004).

There is evidence that urban settings are associated with schizophrenia. Individuals born in the city tend to have around a twofold increase in odds of developing a psychotic condition compared with those born in the rural regions (Marcelis et al. 1998, Mortensen et al. 1999). A recent Irish prospective study confirmed these results: the incidence of schizophrenia was higher in urban areas than in rural areas, with an age-adjusted incidence rate ratio of 1.92 (1.52–2.44) for males and 1.34 (1.00–1.80) for females (Kelly et al. 2010). Migrant status has also been identified as a risk factor for schizophrenia. Compared to native-born individuals, in most but not all studies, immigrants had increased rates of about 2.7-fold (Cantor-Graae and Selten 2005). It has been speculated that infectious etiologies, vitamin deficiencies, or drug abuse might account for this effect (Weiser et al. 2005), but there is no convincing evidence that any of these explanations could be totally responsible for this phenomenon.

Several studies have shown that cannabis use is a risk factor for schizophrenia. Prior cannabis use increases the risk of later schizophrenia about twofold (Zammit et al. 2002, Weiser et al. 2003, Arseneault et al. 2004, Henquet et al. 2005, Semple et al. 2005). In early adolescence the effect size of cannabis use is even larger (Cannon and Clarke 2005). While many investigators believe that cannabis abuse lies along a causal pathway to psychosis (Arseneault et al. 2004), other researchers have found an alternative explanation of this with possible structural and functional impairments in the endogenous cannabinoid system in schizophrenia unrelated to cannabis use (Dean et al. 2001, Leroy et al. 2001, Ujike et al. 2002, De Marchi et al. 2003, Giuffrida et al. 2004, Zavitsanou et al. 2004), so it might be that cannabis smoking is a marker of later schizophrenia.

## 2.7 Etiological theories in schizophrenia

The etiology of schizophrenia has not been clearly understood, although several hypotheses have been developed (Pérez-Neri et al. 2006). In the 1980s several research groups began to speculate that schizophrenia might have a significant developmental component (Murray and Lewis 1987, Weinberger 1987). They were supported by neuropathological studies, which implicated anomalies in early brain development such as aberrant migration of neurons (Church et al. 2002). On the other hand, in the latter part of the 1990s several studies showed evidence of neurodegeneration supporting Kraepelin's original concept of schizophrenia as a degenerative disease

(Arnold et al. 1996, Harrison 1999). The third main biological hypothesis of schizophrenia suggests a dopamine imbalance in the central nervous system (CNS) and is based on pharmacologic studies of antipsychotic drugs first performed by Arvid Carlsson in the 1960s (Carlsson and Lindqvist 1963).

### 2.7.1 Neurodevelopmental hypothesis

In a simple form, the neurodevelopmental hypothesis of schizophrenia suggests that the disease is the behavioural outcome of aberration in neurodevelopmental processes that begins long before the onset of clinical symptoms and is caused by a combination of environmental and genetic factors (Cardno et al. 1999, Singh et al. 2004). With heritability around 80%, nongenetic factors are also an essential part of this model (Rapoport et al. 2005). For more than two decades, the neurodevelopmental hypothesis has been the prevailing explanatory theory for schizophrenia (Rapoport et al. 2005). Initial postmortem studies appeared to support the early neurodevelopmental model with pre- or perinatal lesions as they reported abnormalities in neuronal migration and organisation, considered fetal in origin (Jakob and Beckmann 1986, Akbarian et al. 1993). However, later studies have shown reduced neuronal size and arborisation (Selemon and Goldman-Rakic 1999), which could have developed later in life. This indicates that the pathophysiological processes involved in schizophrenia need not be restricted to the pre- or perinatal period. The evidence that abnormal brain development contributes to schizophrenia comes from several domains, including: a) abnormalities of early motor and cognitive development and histories of obstetrical adversity; b) absence of evidence of neurodegeneration in postmortem tissue studies; and c) association of developmental pathological conditions with adult emergence of psychosis and related phenomena in animal and neurological models (Miyamoto et al. 2003, Schiffman et al. 2009). Schizophrenia may be associated with a subtle, static brain lesion that is caused by a combination of genetic and environmental factors and that eventually interacts with normal maturational processes of the brain to facilitate symptoms such as psychosis (Miyamoto et al. 2003). There is increasing evidence that environmental factors (such as obstetric complications) interact with genetic factors. For example, fetal hypoxia predicts reduced grey matter and increased cerebrospinal fluid volume bilaterally throughout the cortex in schizophrenic patients and their siblings, but not in comparison to subjects at low genetic risk of schizophrenia (Cannon et al. 1993).

Johnstone et al. (1976) first reported dilatation of the lateral ventricles in a small group of patients with chronic schizophrenia. This CT finding has since then been followed by a large number of CT and MRI studies, which have shown enlargement of the lateral and third ventricles in schizophrenia. A median 40% increase in ventricular size was reported in a systematic review of volumetric MRI studies by Lawrie and Abukmeil (1998). The ventricular enlargement is accompanied by a loss of brain tissue averaging 3% (Lawrie and Abukmeil 1998). Larger reductions are seen in volumetric MRI studies in the temporal lobe overall (about 8%) and in medial temporal structures (hippocampus, parahippocampal gyrus, and amygdala, 4–12%) (Lawrie and Abukmeil 1998). Grey matter appears to be reduced more than white matter (Zipursky et al. 1998). Two MRI imaging studies of subcortical structures have shown that the thalamus is smaller in schizophrenia than in healthy controls (Andreasen et al. 1994, Buchsbaum et al. 1996). In a recent MRI imaging study the existence of a midline structure, adhesio interthalamica, was associated with the size of amygdala in schizophrenic patients but not in controls (Takahashi et al. 2008). Knowledge of the timing of the brain changes is essential for understanding their etiological significance. Several studies have shown that volumetric changes are seen in first-episode and medication-free patients (Gur et al. 1998, Whitworth et al. 1998, Zipursky et al. 1998). In addition, adolescents and young adults who are at high risk of developing schizophrenia because of their positive family history show enlarged ventricles (Cannon et al. 1993) and smaller medial temporal lobes (Lawrie et al. 1999). These findings strengthen the assumption that the neuropathological observations are not merely a consequence of the illness and its treatment, and support a neurodevelopmental model of schizophrenia. However, a recent meta-analysis of longitudinal MRI studies showed that lateral ventricular volume continues to increase in patients with schizophrenia after illness onset, which challenges an exclusively neurodevelopmental theory (Kempton et al. 2010).

The most intriguing and potentially most notable histological observations in schizophrenia are those of aberrantly located or clustered neurons, especially in lamina II of the entorhinal cortex (Falkai et al. 2000), and in the neocortical white matter (Kirkpatrick et al. 1999, Eastwood and Harrison 2003) because these kinds of abnormality are strongly indicative of an early neurodevelopmental anomaly affecting neuronal migration, survival, and connectivity (Harrison 1997). Other studies have also found disturbances of neuronal migration in schizophrenia (Jakob and Beckmann 1986, Akbarian et al. 1993, Akbarian et al. 1996).

Histological aberrations have also been seen in the cell bodies (Pierri et al. 2001, Sweet et al. 2003) and dendritic spines and arborisations (Glantz and Lewis 2000, Black et al. 2004) of pyramidal neurons in the hippocampus and neocortex, synaptic projections of interneurons (Lewis 2000a), thalamus (Byne et al. 2002, Danos et al. 2003), and oligodendrocytes (Hof et al. 2003) of schizophrenic subjects. These morphometric changes could contribute to decreased regional brain volumes and cortical thickness, via a reduced neuropil (Selemon and Goldman-Rakic 1999). This means that the schizophrenic cortex contains the same number of neurons as the normal cortex; reduced cortical volume is due to a decrease in interneuronal neuropil, dendritic trees, and cortical afferents (Selemon and Goldman-Rakic 1999).

### 2.7.2 Dopamine hypothesis

The original dopamine hypothesis of schizophrenia formulated by Arvid Carlsson proposed that hyperactivity of dopamine transmission is responsible for the positive symptoms (hallucinations, delusions) observed in this disorder (Carlsson and Lindqvist 1963). This hypothesis was supported by a correlation between clinical doses of antipsychotic drugs and their potency to block dopamine D2 receptors (Seeman and Lee 1975, Creese et al. 1976), and by the psychotogenic effects of dopamine-enhancing drugs (Lieberman et al. 1987).

On the other hand, negative and cognitive symptoms of schizophrenia are generally resistant to treatment by antipsychotic drugs (Keefe et al. 1999). Functional brain imaging studies suggest that these symptoms might be associated with a dysfunction of the prefrontal cortex (Knable and Weinberger 1997). Studies in non-human primates demonstrated that a deficit in dopamine transmission in the prefrontal cortex and lack of stimulation of D1 receptors induce cognitive impairments reminiscent of the ones observed in patients with schizophrenia (Goldman-Rakic et al. 2000). Together, these observations suggest that a deficit in dopamine transmission at D1 receptors in the prefrontal cortex might be implicated in the cognitive impairments and negative symptoms presented by these patients.

Thus, the current view on dopamine and schizophrenia proposes that schizophrenia might be associated with a dopaminergic imbalance, involving an excess of subcortical dopamine function and a deficit in cortical dopamine function. It seems that subcortical mesolimbic dopamine projections are hyperactive, resulting in hyperstimulation of D2 receptors and positive symptoms, while mesocortical

dopamine projections to the prefrontal cortex are hypoactive, leading to hypostimulation of D1 receptors, negative symptoms and cognitive impairment. This view is commonly referred to as the "revised" dopamine hypothesis of schizophrenia (Davis et al. 1991).

Despite decades of effort to generate experimental data supporting these hypotheses, documentation of abnormalities of dopamine function in schizophrenia has remained elusive. The absence of data supporting the dopamine hypothesis of schizophrenia might be a result of the difficulty in obtaining a direct measurement of dopamine transmission in the living human brain. Over the last few years, progress in brain imaging methods has enabled direct measurement of dopamine transmission at D2 receptors, and the application of these techniques to the study of schizophrenia has provided new insights into the nature and the role of dopamine function aberrations in this illness.

### 2.7.3 Neurodegenerative hypothesis

An aberrant neurodevelopment may explain the morphological alterations in the brains of schizophrenic patients but does not explain that at least some of those alterations show a progressive course (Harrison 1999, Lieberman et al. 2001a), which suggests an active neurodegenerative process. Lieberman et al. (2001a) confirmed in their study the earlier findings of ventricular enlargement and anterior hippocampal volume reductions in first-episode schizophrenia patients. In addition, they found increases over time in ventricular volume in poor-outcome patients referring to a continuing pathological process in this patient group. In a recent 4-year longitudinal MRI study using voxel-based morphometry, Mané et al. (2009) detected an excessive decrease in grey matter in patients as compared to healthy controls in the left superior temporal gyrus and right orbitofrontal gyrus, and an excessive increase in the bilateral lingual gyrus and right cuneus. A general decrease of brain mass from the onset of the disorder has also been reported (DeLisi et al. 1997, Gur et al. 1998, Rapoport et al. 1999).

One of the main arguments against the neurodegenerative hypothesis in schizophrenia has been that most recent histopathologic studies have failed to find gliosis in the brains of schizophrenic patients (Falke et al. 2000, Popken et al. 2000, Damadzic et al. 2001). However, opposite results have also been reported in an earlier study (Stevens 1982). Apoptotic mechanisms have been suggested to

occur in schizophrenia (Jarskog et al. 2005). Apoptotic cell death proceeds without incurring a gliotic response (Jarskog et al. 2005). Significantly reduced content of the antiapoptotic protein Bcl-2 (Jarskog et al. 2000) and an increased Bax/Bcl-2 ratio (associating with susceptibility for apoptotic death) in Brodmann´s area 21 (Jarskog et al. 2004) and ultrastructural changes in oligodendroglial cells suggestive of apoptotic death (Uranova et al. 2001) have been found in the brains of people who died with schizophrenia. There is evidence that apoptosis can also be localised to synapses or distal neurites without inducing immediate neuronal death (Mattson et al. 1998). This makes apoptosis interesting because neuropathological studies in schizophrenia have shown evidence of shorter dendrites, reduced neuropil, limited reductions in neuronal and glial cell numbers, a lack of gliosis, and in vivo neuroimaging evidence of progressive grey matter loss early in the disorder.

### 2.7.4 Excitotoxic (glutamatergic) hypothesis

The excitotoxic hypothesis of schizophrenia suggests that there is, at least, a subtype of patients with schizophrenia in which progressive excitotoxic neuronal cell death in hippocampal and cortical areas occurs via disinhibition of glutamatergic projections to these areas (Deutsch et al. 2001). These patients are expected to have poor outcomes characterised by pronounced negative symptoms and cognitive deficits, and profound psychosocial deterioration (Deutsch et al. 2001). According to this theory, N-acetyl-D-aspartate receptors in gamma-aminobutyric acid (GABA)-ergic inhibitory interneurons in the cortex and hippocampus are antagonised, which leads to disinhibition of downstream glutamatergic projections, whereby resulting in excessive release of glutamate and a potential cascade of excitotoxic events, including the generation of reactive oxygen species and apoptotic cell death (Deutsch et al. 2001). Mitochondrial dysfunction and deficiencies in energetic metabolism, which have also been suggested to occur in schizophrenia (Ben-Schachar 2002), may sensitise neurons to the physiological glutamate concentrations in the extracellular fluid, leading to excitotoxic cell death (Kornhuber and Weller 1997). Reduced expression of brain-derived neurotrophic factor (BDNF) and its receptor (Hashimoto et al. 2005a) could also contribute to an enhanced susceptibility to apoptotic cell death in schizophrenia, since this neurotrophin possesses neuroprotective properties (Morse et al. 1993). Reduced BDNF content has been reported in the prefrontal cortex (Weickert et al. 2003, Hashimoto et al. 2005a) from schizophrenic brains

and reduced (Toyooka et al. 2002) or unchanged (Shimizu et al. 2003) in serum from schizophrenic patients. Glutamatergic hypofunction is suggested to have a role in decreased BDNF expression because glutamate antagonist kynurenic acid blocks the increased mRNA BDNF content induced by the activation of glutamatergic receptors in hippocampal neurons (Zafra et al. 1990).

In conclusion, the etiology of schizophrenia remains unknown, but growing evidence suggests that it might involve two pathophysiologic processes: one early in life (neurodevelopmental), and one after the onset of the illness (neurodegenerative) (Mané et al. 2009, Tanskanen et al. 2010). A unifying hypothesis has been formulated that conceptualises schizophrenia as a progressive neurodevelopmental disorder (Gupta and Kulhara 2010, Rapoport and Gogtay 2011).

## 2.8 Genetics of schizophrenia

Twin studies have clearly shown that schizophrenia is predominantly a genetic disorder. Heritability is defined as the proportion of phenotypic variance attributable to genetic variance. Studies have shown that the heritability is around 80% in schizophrenia (Cardno and Gottesman 2000, Sullivan et al. 2003). Family studies show that simple major gene effects are unlikely; instead, polygenic models, that is, the effects of multiple risk genes, provide the best explanatory fit (Gottesman and Shields 1967). Schizophrenia seems to be a complex genetic disorder, not characterised by a single causative gene and not showing simple patterns of inheritance (Harrison and Weinberger 2005). It is likely that the risk genes of schizophrenia are modified by other genes, including protective ones, both additively and epistatically, and also show environmental modification (Harrison and Weinberger 2005).

### 2.8.1 Family, twin and adoption studies

Family, twin and adoption studies are the primary means by which the genetic basis of a disorder may be established. Family studies sample affected individuals and assess the risk of the disorder in their relatives. Family studies can determine familiarity of a disorder but cannot verify whether the aggregation within families is due to genetics or the environment because family members generally share both types of influences. Twin and adoption studies are used to differentiate genetic from shared

environmental factors. Twin studies compare the rate of agreement of a disorder between identical or monozygotic (MZ) twins and dizygotic (DZ) twins. Because MZ twins share 100% of their genes and DZ twins share 50% of their genes on average, the concordance rate for a genetically influenced disorder is expected to be higher for MZ twins than for DZ twins. Adoption studies examine the shared environment of non-genetically related family members and the shared genetics of family members who have dissimilar family environments. Studies of adoption can therefore help to distinguish the relative influence of genes and the environment. A high relative risk of a disorder in both adoptees and adoptive parents suggests shared environmental influence, whereas similar risks in adoptee and biological parents suggest genetic influences.

Family studies have consistently shown that schizophrenia clusters in families. In various family studies, first-degree relatives of schizophrenic patients have had a higher morbidity risk of schizophrenia of about 2–9% compared to the risk in relatives of controls (0–1%) (Shih et al. 2004). Studies have also found an increased risk in second- and third-degree relatives with risk estimates of 3% and 1.5%, respectively (Maier et al. 2002).

Twin studies indicate that there is a genetic component to the etiology of schizophrenia, as several studies have shown MZ twin concordance rates to be higher than DZ rates. Kendler (1983) found in his review of twin studies conducted before 1980 a pooled estimate of concordance for MZ twins of 53% and for DZ twins of 15%. In a review of six more recent twin studies, Cardno et al. (1999) reported a pooled concordance estimate of 50% for MZ twins and 4.1% for DZ twins.

Adoption studies have found that adopted-away children of mothers with schizophrenia have a higher risk of schizophrenia than adoptees with biological mothers who do not have schizophrenia (Lowing et al. 1983, Tienari et al. 2000, Tienari et al. 2003). In the Danish adoptee study there were three times more schizophrenia patients in adoptees that had had a psychotic parent than in adoptees whose biological parents had never had a psychosis diagnosis (Lowing et al. 1983). Tienari et al. (2000) found that lifetime prevalence of typical schizophrenia in 164 index adoptees was 6.7% (8.1% age-corrected morbid risk), which differed significantly from the 2.0% prevalence (2.3% age-corrected morbid risk) in 197 control adoptees. Tienari et al. (2003) compared 190 adoptees whose mothers had schizophrenia spectrum disorders, to 192 low-risk adoptees whose biological mothers had either a non-schizophrenia spectrum diagnosis or no lifetime psychiatric diagnosis. They detected that the mean age-corrected morbid risk of a schizophrenia spectrum disorder was

significantly higher in adoptees whose mothers had schizophrenia spectrum disorders (22.46%) than in low-risk adoptees (4.36%). In this study the morbid risk of schizophrenia in the Finnish adoptees whose biological mothers had schizophrenia was 5.34%, whereas in previous studies with offspring reared by biological parents with schizophrenia the risk has been found to be considerably higher, from 11.1% (Erlenmeyer-Kimling et al. 1995, Erlenmeyer-Kimling et al. 1997) to 16.2% (Parnas et al. 1993). Tienari et al. (2003) suggest that this difference could be attributed to protective rearing by the adoptive parents. Kendler et al. (1994) reported that biological relatives of affected adoptees had a higher risk of a schizophrenia spectrum disorder than adoptive relatives (13.0% vs. 1.2%, respectively). In addition, the risk in adoptive relatives of developing schizophrenia was similar to the overall risk in the general population.

### 2.8.2 Linkage studies

Family, twin and adoption studies have shown that schizophrenia is predominantly a genetic disorder. Several linkage studies with genome-wide scans have been conducted in the last two decades and significant linkages have been found. Hovatta et al. (1999) performed a genome-wide screen for susceptibility genes for schizophrenia in a Finnish internal genetic isolate and detected positive results for the schizophrenia trait on chromosomal regions 1q32.2-q41, 4q31, 9q21, and Xp11.4-p11.3. A Finnish population-wide four-stage genome scan of 134 affected sib-pairs provided evidence for linkage to chromosome 7q22 and some additional support for linkage to chromosome 1q32.2-q41 (Ekelund et al. 2000). Paunio et al. (2001) reported linkage of schizophrenia or schizophrenia spectrum conditions to chromosomes 2q, 5q and 1q in a population-wide genome-wide scan of a sample of 1265 individuals from 238 Finnish pedigrees. In one of the largest complete genome-wide scans of schizophrenia with 382 sibling pairs with a diagnosis of schizophrenia or schizoaffective disorder, DeLisi et al. (2002) found a significant linkage in chromosome 10p14 and a suggestion of linkage in chromosome 2p14-q12. In a meta-analysis by Badner and Gershon (2002), three loci reached genome-wide significance: 8p, 13q, and 22q, which the authors considered valid linkage regions, likely containing one or more susceptibility genes. Lewis et al. (2003) performed a meta-analysis of 20 genome scan studies and found genome-wide significance only for chromosome 2p12-22.1, but using a more liberal statistical threshold, several other loci emerged:

5q, 3p, 11q, 2q, 1q, 22q, 8p, 6p, 20p, and 14q. Ng et al. (2009) conducted a large genome scan meta-analysis on 32 independent genome-wide linkage scans including 3255 pedigrees with 7413 genotyped cases with schizophrenia or related disorders. In the primary analysis they found suggestive evidence of linkage on chromosomes 5q and 2q, and in the secondary analysis significant evidence for linkage on chromosome 2q. Ng et al. (2009) also performed a secondary analysis of 22 studies of European-ancestry samples with 1835 pedigrees with 4141 genotyped cases affected by schizophrenia or related disorders, which showed suggestive evidence of linkage on chromosome 8p. However, replication has been inconsistent for reports of significant genetic linkage. There have been many explanations offered for these apparent inconsistencies, including a lack of power to detect the weak linkages expected in complex genetic disorders, and problems with diagnostic precision and genetic heterogeneity (Harrison and Weinberger 2005). The inconsistent results regarding genetic linkage analyses have led to two schools of thought: a sceptical one, which views the strategy as nonproductive, and an optimistic one, which sees the results as being consistent with predictions of weak effects of multiple genes and with genetic heterogeneity.

### 2.8.3 Candidate gene approach

The public genome databases have made it possible for researchers to identify candidate genes or expressed sequences within the linkage regions and, together with available functional information, make a case for gene identification. This has led to testing of specific allelic variants, usually single-nucleotide polymorphisms (SNPs), within or adjacent to a gene (Harrison and Weinberger 2005). Allelic frequencies of the SNP are compared between schizophrenic patients and healthy controls and if there is a significant difference between patients and controls, a genetic association is said to exist. Such an association, if not an artefact (caused by, for example, multiple testing, population stratification, or genotype errors), means that the SNP is either causative or is in linkage disequilibrium (LD) with a causative variant. An important point is, however, that eventually identifying susceptibility genes for polygenic disorders like schizophrenia will require biological evidence that the risk variant impacts on the pathogenesis of the disease (Weiss and Terwilliger 2000, Page et al. 2003).

### 2.8.3.1 Interleukin-1 gene complex

Interleukin-1 (IL-1) is a pleiotropic cytokine that functions in the context of inflammation, cell growth and tissue repair (Vamvakopoulos et al. 2002). IL-1 is proinflammatory, influences neurodegenerative and neuroprotective processes in the brain, and is involved in the modulation of synaptic plasticity (Rothwell and Luheshi 2000). IL-1 exists in at least two isoforms: IL-1α and IL-1β with a competing IL-1 receptor antagonist (IL-1RA). The interleukin-1 cluster is located on chromosome 2q13-21, which has been linked to schizophrenia in several studies (Levinson et al. 1998, Paunio et al. 2001).

The cytokine hypothesis of schizophrenia proposes that cytokines transmit peripheral inflammatory signals to immature brain tissue through the developing blood-brain-barrier perturbing structural and phenotypic development of the brain (Watanabe et al. 2010). This provides an example of gene-environment interaction in the vulnerability to schizophrenia: various environmental factors, such as inflammation or infection, cause activation of inflammatory cytokine production, which is hypothesised to be genetically aberrant in people vulnerable to schizophrenia, leading to disturbed development of the brain. Numerous studies have provided evidence of abnormal levels of cytokines of the IL-1 cluster in the peripheral blood of schizophrenic patients, indicating dysregulation of these cytokines in schizophrenia (Katila et al. 1994, Akiyama 1999, Theodoropoulou et al. 2001). It has also been demonstrated that IL-1 activation during prenatal infections and perinatal hypoxia may be related to schizophrenia (Buka et al. 1993, Gilmore and Jarskog 1997, Orzylowska et al. 1999, Rosso et al. 2000, Zornberg et al. 2000, Limosin et al. 2003). Human studies suggest that there is an imbalance between IL-1 and IL-1RA, with a shift towards IL-1 signalling in the brains of schizophrenic patients (Watanabe et al. 2010). Animal models have shown that neonatal treatment with IL-1α produces the long-lasting schizophrenia-like behavioural abnormalities, some of which can be alleviated by second-generation antipsychotics, providing further support for this hypothesis (Watanabe et al. 2010).

There are several studies which show that the allele variation in the IL-1-gene cluster has a clear impact on the levels of these cytokines. The gene encoding IL-1RA (IL-1RN) has a variable number of tandem repeat polymorphisms (VNTR, rs380092) in intron 2 (Tarlow et al. 1993). In the IL-1B gene there is an SNP (rs16944) at the promoter region at position -511 (di Giovine et al. 1992), and in the IL-1A gene there is an SNP (rs1800587) at the promoter region at position -889 (McDowell

et al. 1995). In a transcriptional study the IL-1B-511 (rs16944) allele 2 was associated with increased transcriptional activity, but only in the context of the IL-1B-31 polymorphism (Chen et al. 2006). Hurme and Santtila (1998) showed that the IL-1RN allele 2 carriers have clearly higher levels of IL-1RA than the non-carriers in healthy blood donors. They also demonstrated that the presence of IL-1B-511 allele 2 has an enhancing effect on plasma IL-1RA levels in IL-1RN allele 2 carriers. In another report the IL-1RN allele 2 was consistently associated with higher IL-1RA and lower IL-1β release (Vamvakopoulos et al. 2002). Hacker et al. (2001) found also that the carriers of the IL-1RN allele 2 had increased concentrations of IL-1RA compared with non-carriers in healthy volunteers. There is also evidence that in healthy blood donors, IL-1A-889 allele 2 homozygotes had significantly elevated levels of IL-1β and that the finding was even more pronounced in donors who also were carriers of the IL-1B allele 2 (Hulkkonen et al. 2000). When combining the above-mentioned findings we can assume that the allele combination of IL-1A allele 2, IL-1B allele 1, and IL-1RN allele 1, especially in homozygote form, produces an elevated IL-1β/IL-1RA ratio with a high proinflammatory IL-1β effect. High IL-1β concentration has been shown to decrease neuronal survival in hippocampal and cerebral cortical cultures (Araujo and Cotman 1995, Marx et al. 2001). Thus, this allele combination could have neurotoxic effects on the developing CNS, when the interleukin-1 complex is activated during infection or ischemia.

In a Spanish study a trend for the association between the IL-1B-511 allele 2 and schizophrenia spectrum disorders was found and when using a multidimensional symptom approach to the diagnosis, the association was confirmed in psychotic patients showing the depressive symptom dimension (Rosa et al. 2004). Meisenzahl et al. (2001) investigated the effect of the IL-1β genetic polymorphism on brain morphology in schizophrenia. Their data suggested that schizophrenic patients with the IL-1B-511 allele 2 showed both significantly smaller bifrontal temporal grey matter volumes and generalised white matter tissue deficits compared to the patients homozygous for the IL-1-B-511 allele 1. Papiol et al. (2004) found a significant excess of the haplotypic combination of IL-1B-511 allele 1 and IL-1RN VNTR allele 2 in schizophrenics compared with controls. Zanardini et al. (2003) demonstrated that the frequencies of IL-1B-511 allele 1 and IL-1RN VNTR allele 1 are significantly higher in schizophrenic patients than in controls. On the contrary, Kim et al. (2004) found that the IL-1RN allele 2 was associated with schizophrenia compared with healthy controls in Korean samples. Mata et al. (2006) studied the IL-1RN VNTR polymorphism in Spanish Caucasian schizophrenic patients and

healthy controls and found no differences in genotype or allele frequencies between patients and controls. However, the VNTR polymorphism significantly predicted negative symptom improvement during antipsychotic treatment, i.e. homozygous patients for the IL-1RN allele 2 showed the greatest improvement, followed by heterozygous patients and then homozygous patients for the IL-1RN allele 1 (Mata et al. 2006). Chowdari et al. (2001) studied the IL-1A-889, IL-1B-511 and IL-1RN VNTR polymorphisms with a case-control design in two independent Chinese populations and found no significant differences with respect to allele, genotype or haplotype frequencies between schizophrenic patients and controls. Xu and He (2010) performed a meta-analysis concerning the four most commonly reported SNPs of the IL-1 gene complex and detected that the IL-1-B-511 polymorphism (rs16944) was associated with schizophrenia. Sasayama et al. (2011) analysed five tagging polymorphisms of the IL-1β gene in a population of 533 Japanese patients with schizophrenia and 1136 healthy controls and found an association between the IL-1β polymorphism rs 1143633 and schizophrenia in females. There was also a trend towards an association between rs 16944 and female patients with schizophrenia. Recently, Borkowska et al. (2012) investigated genetic polymorphisms -31T/C (rs1143627) and -511C/T (rs16944) in the IL-1β promoter region in 143 unrelated Caucasian Polish paranoid schizophrenia patients and 206 healthy unrelated individuals and detected significant differences for the -31T/C polymorphism ($P < 0.001$) but no significant differences for the -511C/T polymorphism. However, the copresence of genotypes T/T of -31 and C/C of -511 IL-1β polymorphisms was associated with schizophrenia in their study (Borkowska et al. 2011) (Table 2).

**Table 2.** Association studies of IL-1 gene complex and schizophrenia.

| Polymorphism | Reference | N | Result (P value, if significant) |
|---|---|---|---|
| IL-1A-889 | Chowdari et al. 2001 | 171+100 patients, 130+99 controls | Not significant |
| IL-1B-511 | Chowdari et al. 2001 | 171+100 patients, 130+99 controls | Not significant |
| | Zanardini et al. 2003 | 169 patients, 177 controls | Significant (P=0.047) |
| | Papiol et al. 2004 | 78 patients, 176 controls | Significant in haplotypic combination with IL-1 VNTR (P=0.0015) |
| | Rosa et al. 2004 | 89 patients, 267 controls | Significant in depressed patients (P=0.02) |
| | Sasayama et al. 2011 | 533 patients, 1136 controls | Trend towards association in females (P=0.032; significance level set at P < 0.013) |
| | Borkowska et al. (2012) | 143 patients, 206 controls | Copresence of genotypes T/T of -31 and C/C of -511 polymorphisms associated with paranoid schizophrenia (P value not mentioned) |
| IL-1RN VNTR | Chowdari et al. 2001 | 171+100 patients, 130+99 controls | Not significant |
| | Zanardini et al. 2003 | 169 patients, 177 controls | Significant (P=0.002) |
| | Kim et al. 2004 | 269 patients, 297 controls | Significant (P=0.009) |
| | Mata et al. 2006 | 154 patients, 336 controls | Significant concerning response to antipsychotic drugs (P=0.006) |
| rs1143633 | Sasayama et al. 2011 | 533 patients, 1136 controls | Significant in females (P=0.0089) |

### 2.8.3.2 Tumour necrosis factor alpha

Elevated levels of proinflammatory cytokines like tumour necrosis factor-alpha (TNF-α) have been detected in peripheral blood of schizophrenic patients (Ganguli et al. 1994, Katila et al. 1994, Naudin et al. 1997, Lin et al. 1998, Maes et al. 2000).

TNF-α is secreted in the CNS by neurons, glial cells and astrocytes (Lieberman et al. 1989, Hunt et al. 1992, Merrill 1992, Pan et al. 1997, Mousa et al. 1999). TNF-α receptors have also been identified in the brain, where the greatest specific binding was in the brainstem and was also detected in the cortex, thalamus and basal ganglia, and least in the cerebellum (Kinouchi et al. 1991). TNF-α has been shown to have several functions in the CNS, including nerve-cell growth, differentiation and apoptosis. TNF-α affects the formation of neurites and neuronal survival in the CNS (Marx et al. 2001, Neumann et al. 2002). Continual presence of TNF-α is needed to maintain synaptic strength at excitatory synapses in the brain (Beattie et al. 2002). On the other hand, TNF-α has been demonstrated to have cytotoxic effects on embryonic mesencephalic dopaminergic neurons (McGuire et al. 2001), which are suggested to have an important role in the etiopathogenesis of schizophrenia. There is evidence that astrocytes, immunologically competent glial cells of the CNS, stimulated by TNF-α respond with increased expression of several genes, including chemokines, growth factors, neuromodulin and receptors (Meeuwsen et al. 2003). These various effects suggest that disturbances in the level of TNF-α may affect the functioning of the brain. TNF-α has been demonstrated to have a cytotoxic role in several neurologic disorders, including Alzheimer´s disease, multiple sclerosis, Parkinson´s disease and brain trauma (Shohami et al. 1999, Nagatsu et al. 2000, Killestein et al. 2001, Wenk et al. 2003). The impact of TNF-α in the pathogenesis of schizophrenia is not clear but it has been shown to have a stimulatory effect on the cathecholaminergic system, whereas chronic TNF-α release induces an inhibitory effect (Soliven and Albert 1992). This is in line with the theory that in acute schizophrenia there is a hyperdopaminergic state with positive psychotic symptoms, whereas chronic schizophrenia with predominantly negative symptoms is associated with a relative hypodopaminergic state (Rao and Moller 1994, Lewis and Lieberman 2000b). Another evidence that TNF-α can be implicated in the pathogenesis of schizophrenia comes from linkage studies: the TNF-α gene is located within the MHC region at the short arm of chromosome 6 (6p21.1-21.3), a region that has been linked to schizophrenia in several studies (Wright et al. 1996, Gershon et al. 1998, Schwab et al. 2000). Recent genome-wide association studies have also found a significant association with several markers spanning the MHC region on chromosome 6p21.3-22.1 (International Schizophrenia Consortium 2009, Shi et al. 2009, Stefansson et al. 2009).

The TNF-α gene has several polymorphisms in the promoter region. There is a well-known biallelic base exchange polymorphism at nucleotide position –308

(rs1800629), which consists of two alleles with a more common variant with guanine (G) (-308G) and a less common variant with adenine (A) (-308A) at position –308. This polymorphism has been shown to directly affect the transcription of TNF-α (Kroeger et al. 1997, Wilson et al. 1997, Louis et al. 1998). There is also evidence that the –308A allele is associated with higher TNF-α production (Wilson et al. 1997). However, Brinkman et al. (1995) did not find any difference in the level of transcription between the –308G and –308A alleles in lipopolysaccharide-stimulated peripheral blood monocytes.

There have been several recent studies concerning the possible association between the TNF-α -G308A polymorphism and schizophrenia. In the first study, Boin et al. (2001) found that the frequency of the –308A allele was significantly increased in Caucasian Italian schizophrenic patients as compared with controls with the same ethnic background. Genotype distribution was also significantly different between schizophrenics and controls in their study and –308A homozygotes were represented only in the patient group. The results were confirmed in a Brazilian population (Meira-Lima et al. 2003). By contrast, there are three novel studies showing increased frequency of the –308G allele in schizophrenic patients as compared with controls in Caucasian German, Chinese Singaporean and Polish population groups (Schwab et al. 2003b, Tan et al. 2003, Czerski et al. 2008). In several studies there has not been any detected association between the TNF-α -G308A polymorphism and schizophrenia (Riedel et al. 2002, Handoko et al. 2003, Tsai et al. 2003, Duan et al. 2004, Hashimoto et al. 2004, Pae et al. 2006, Watanabe et al. 2007a). The subjects in these studies were of Caucasian and Han Chinese origin (Riedel et al. 2002, Tsai et al. 2003), Japanese origin (Hashimoto et al. 2004, Watanabe et al. 2007a), Korean origin (Pae et al. 2006), and from four distinct populations in the Asia-Pacific region (Handoko et al. 2003). A haplotype analysis of the –G308A and -G238A polymorphisms of the TNF-α gene in 24 Canadian families of primarily Celtic origin showed evidence of an association of a specific haplotype (-308A, -238G) with schizophrenia and schizophrenia spectrum disorders (Saviouk et al. 2005). Zai et al. (2006) did a family and case-control study in Canada and found no association between the TNF-α -G308A polymorphism and occurrence of schizophrenia. However, patients with allele A showed significantly better treatment response to clozapine medication when compared with patients without allele A (Zai et al. 2006). Shirts et al. (2006a) performed a genotype and haplotype analysis of 8 SNPs in the TNF-α gene including –G308A in schizophrenia and schizoaffective patients with Caucasian ancestry and found no association with schizophrenia.

Naz et al. (2011) performed a case-control study of the TNF-αG308A polymorphism in a Pakistani population and found the homozygous A/A genotype more frequently in schizophrenia patients than in healthy controls. Thus, the results are highly controversial, suggesting possible ethnic differences in the genotype and allele distributions between schizophrenic patients and healthy controls in the TNF-α -G308A (rs1800629) polymorphism (Table 3).

**Table 3.** Association studies of tumour necrosis factor alpha (TNF-α) -G308A polymorphism and schizophrenia.

| Polymorphism | Reference | N | Result (P value, if significant) |
|---|---|---|---|
| TNF-α -G308A | Boin et al. 2001 | 84 patients, 138 controls | Significant (P=0.0042) |
| | Riedel et al. 2002 | 157 patients, 186 controls | Not significant |
| | Handoko et al. 2003 | 390 patients, 803 controls | Not significant |
| | Meira-Lima et al. 2003 | 186 patients, 657 controls | Significant (P=0.01) |
| | Schwab et al. 2003b | 207 families (sib pairs and trios combined), 300 affected offspring | Significant (P=0.003) |
| | Tan et al. 2003 | 302 patients, 152 controls | Significant (P<0.001) |
| | Tsai et al. 2003 | 205 patients, 192 controls | Not significant |
| | Duan et al. 2004 | 340 patients, 314 controls | Not significant |
| | Hashimoto et al. 2004 | 297 patients, 458 controls | Not significant |
| | Saviouk et al. 2005 | 24 families, 330 subjects | Significant in haplotype with -G238A (P=0.026) |
| | Pae et al. 2006 | 152 patients, 152 controls | Not significant |
| | Shirts et al. 2006a | 244 patients, 276 controls | Not significant |
| | Zai et al. 2006 | 95 patients, 95 families<br>149 patients, 149 controls | Not significant<br>Not significant |
| | Watanabe et al. 2007a | 265 patients, 424 controls<br>83 trios | Not significant<br>Not significant |
| | Czerski et al. 2008 | 348 patients, 351 controls | Significant (P=0.008) |
| | Naz et al. 2011 | 100 patients, 70 controls | Significant (P=0.015) |

### 2.8.3.3 Epidermal growth factor

Epidermal growth factor (EGF) is a polypeptide, which stimulates the proliferation of ectodermal and mesodermal cells (Carpenter and Cohen 1979). It is a member of the EGF family with structurally related factors, such as transforming growth factor (TGF) α and heparin-binding EGF-like growth factor (HB-EGF). Lazar and Blum (1992) have shown EGF production in as early as embryonic day 14 in a mouse brain and it could be detected until the postnatal period in the whole brain. They found EGF mRNA in all regions examined, including the brainstem, cerebellum, cerebral cortex, hippocampus, basal hypothalamus, olfactory bulb, striatum and thalamus. Also, HB-EGF mRNA and TGF-α mRNA have widespread distribution in the brain in neurons and glial cells (Seroogy et al. 1993, Nakagawa et al. 1998). EGF receptors (EGFR) have been detected in the brain during the fetal period (Adamson and Meek 1984). Seroogy et al. (1995) have shown with an in situ hybridisation technique that EGFR is expressed in the forebrain ventricular/subventricular zone and cerebellar external granule layer, which are principal germinal zones of a developing rat brain, suggesting that EGF regulates the activities of neuronal and glial progenitor cells in the developing brain in vivo.

EGF acts as a mitogen on neuronal progenitor cells in the CNS and stimulates the proliferation of neuronal precursor cells in various parts of the CNS (Anchan et al. 1991, Reynolds et al. 1992, Santa-Olalla and Covarrubias 1995). EGF also has neurotrophic properties on several postmitotic neurons of the CNS (Plata-Salamán 1991). EGF has been shown to have various functions on the growth and survival of midbrain dopaminergic neurons (Casper et al. 1991, Ferrari et al. 1991, Pezzoli et al. 1991, Casper et al. 1994, Farkas and Krieglstein 2002). EGF exerts not only neurotrophic effects but also neuromodulatory effects on neurons in the CNS (Plata-Salamán 1991). EGF has been shown to increase the long-term potentiation in synapses of hippocampal neurons (Terlau and Seifert 1989) and to enhance the N-methyl-D-aspartate (NMDA) receptor-mediated increase of the intracellular Ca2+ concentration in hippocampal neurons (Abe and Saito 1992). Therefore, EGF may be related to the regulation of synaptic plasticity in the neurons of the hippocampus (Yamada et al. 1997). EGF decreases the neurotransmitter glutamate release in forebrain synaptosomes (Barrie et al. 1996).

As explained earlier, the developmental model of schizophrenia suggests that the changes in the CNS, which sensitise a person to schizophrenia, have their origin already in the fetal period or later during the development of CNS. Dopaminergic

dysfunction is thought to have a role in the etiology of schizophrenia (Goldstein and Deutch 1992, Kerwin 1993, Seeman 1993, Weinberger 1997). EGF family peptides exert neurotrophic and neuromodulatory effects on developing dopaminergic neurons in vitro and in vivo (Casper et al. 1991, Ferrari et al. 1991, Plata-Salamán 1991, Alexi and Hefti 1993). They have also been shown to have neuroprotective properties in dopaminergic neurons in vitro (Casper and Blum 1995). Futamura et al. (2002) have studied EGF family protein levels in postmortem schizophrenic brains compared to control subjects and found that EGF levels were decreased in the prefrontal cortex and striatum of schizophrenic patients, whereas no significant differences were detected in the levels of HB-EGF and TGFα between schizophrenics and controls in any regions examined. They also found elevated EGFR expression in the prefrontal cortex in schizophrenic patients (Futamura et al. 2002).

Shahbazi et al. (2002) studied the possible association of malignant melanoma and genetic polymorphisms of the EGF gene and found that there is a single nucleotide polymorphism (SNP) (G to A) at position 61 (rs4444903) in the EGF gene. They also found that in vitro cells from 61A homozygous individuals produced significantly less EGF than cells from G/G or G/A individuals (Shahbazi et al. 2002). Several studies of the genetic association between schizophrenia and this polymorphism were subsequently conducted. Anttila et al. (2004) found a positive association: the G allele was more common in Finnish male patients with schizophrenia than in male controls. Later studies with Japanese and Korean samples have not confirmed this result (Lim et al. 2005, Watanabe et al. 2005, Lee et al. 2006).

Anttila et al.´s (2004) study indicated that the G allele of EGF (rs4444903) was associated with the early onset of schizophrenia in male patients. Lee et al. (2006) also found a gender difference in EGF polymorphisms: male patients with early-onset schizophrenia were more likely to exhibit the common AA homozygote than male patients with adult-onset schizophrenia. Kampman et al.´s (2005) study revealed an interaction between the EGF and TNF-α polymorphisms concerning the age of onset of schizophrenia: patients with the EGF AA and TNF-α –G308A AG/AA genotype had a lower age of onset than the rest of the patients not having this combination (Table 4).

**Table 4.** Association studies of epidermal growth factor (EGF) A61G polymorphism and schizophrenia.

| Reference | N | Result (P value, if significant) |
|---|---|---|
| Anttila et al. 2004 | 94 patients, 98 controls | Significant in male patients (P=0.008) |
| Kampman et al. 2005 | 94 patients, 98 controls | Significant interaction with EGF and tumour necrosis factor-alpha in age of onset of schizophrenia (P<0.001) |
| Lim et al. 2005 | 174 patients, 132 controls | Not significant |
| Watanabe et al. 2005 | 337 patients, 421 controls | Not significant |
| Lee et al. 2006 | 190 patients, 347 controls | Significant gender difference: early-onset males are more often AA homozygotes than adult-onset males (P=0.030), not observed in females |

### 2.8.3.4 Dopamine-2 receptor

Dopamine receptors were originally classified into two types: the D1 receptors, stimulating adenylate cyclase; and the D2 receptors, not coupled to or inhibiting this effector (Kebabian and Calne 1979). Later, three new dopamine receptors — D3, D4, and D5 — were found (Sokoloff et al. 1990, Sunahara et al. 1991, Tiberi et al. 1991, Van Tol et al. 1991). Pharmacological studies of these receptors showed that D1 and D5 shared similar properties, whereas the pharmacological profiles of D3 and D4 resembled the D2 type. Therefore, dopamine receptors are now divided into two groups: the D1-like family (D1 and D5 receptors) and the D2-like family (D2, D3 and D4 receptors). D2 receptors are both postsynaptic receptors and presynaptic autoreceptors (Laruelle 2003a). D2 receptors locate mainly in the striatum, with low concentration in medial temporal structures (hippocampus, entorhinal cortex, amygdala) and the thalamus (Laruelle 2003a). In the striatum, D2 receptors are preferentially found in encephalin-rich GABAergic neurones (Le Moine et al. 1990). The action of dopamine (DA) on target neurones is neither "inhibitory" nor "excitatory", but its action will depend on the state of the neurones at the time of the stimulation (Yang et al. 1999). In the striatum, it has been proposed that DA is augmenting the inhibition of GABAergic neurones that are inhibited and the excitability of neurones that are excited. In this way, DA acts to gate glutamatergic inputs by increasing their signal/noise ratio (Laruelle 2003a). In addition, DA input might produce long-term changes in the strength of corticostriatal glutamatergic synapses (long-term depression, LTD, and long-term potentiation, LTP) (Arbuthnott et al. 2000, Kerr and Wickens 2001), a process which might have a role in

the plasticity associated with the emergence of positive symptoms upon prolonged DA hyperactivity.

Several lines of evidence implicate the dopamine D2 receptor (DRD2) gene as a candidate gene for susceptibility to schizophrenia. The dopamine hypothesis of schizophrenia suggests that subcortical mesolimbic dopamine projections are hyperactive, resulting in hyperstimulation of D2 receptors and positive symptoms. The current antipsychotic medications for schizophrenia consist of either full or partial antagonists of the DRD2 (Meltzer 2004, Miyamoto et al. 2005). Additionally, neuroimaging evidence indicates that schizophrenic patients have increased brain dopamine D2 receptor density (Seeman and Kapur 2000). For example, untreated patients with schizophrenia and controls pharmacologically depleted of endogenous dopamine have been studied using in vivo measurements of D2 receptor availability made both before and after dopamine depletion (Abi-Dargham et al. 2000). The schizophrenic subjects demonstrated a larger increase in D2 receptor availability post-dopamine depletion than controls. These data suggest that there is a physiological state of increased D2 receptor availability in schizophrenia. There are several DRD2 gene polymorphisms; the three most actively studied for a possible role in schizophrenia are the TaqI A1/A2 SNP (rs1800497), the deletion of cytosine at position 141 in the promoter region (-141 Ins/Del), and the C960G missense polymorphism (rs1801028) that leads to a Ser0Cys substitution at position 311 at the protein level (Dubertret et al. 2004). For the most part, previous studies investigating the association of these polymorphisms with schizophrenia have reported negative findings or have not successfully been replicated; the most consistent association has been found between the TaqI A1/A2 polymorphism and schizophrenia occurrence and age of schizophrenia onset (Dubertret et al. 2001, Dubertret et al. 2004) (Table 1). In addition, a meta-analysis concerning all published case-control studies of the DRD2 Ser311Cys (rs1801028) polymorphism showed a significant association between the Cys311 allele and schizophrenia (Jönsson et al. 2003).

A synonymous polymorphism in the DRD2 gene, the C957T (rs6277) is in linkage disequilibrium with Taq 1A (Duan et al. 2003). In vitro studies have shown that the DRD2 C957T polymorphism has marked functional consequences for DRD2 mRNA stability and dopamine-regulated DRD2 expression: the T allele of this SNP is associated with decreased mRNA translation and stability, while the C allele is not (Duan et al. 2003). Duan et al. (2003) suggest that these differences may be due to changes in the predicted mRNA secondary structure. In addition, Hirvonen et al. (2004) have reported that C957T affects striatal D2 binding in

healthy humans. Their studies suggest that striatal D2 binding is highest in T/T homozygotes, lowest in C/C homozygotes, and intermediate in C/T heterozygotes. However, recent in vivo investigations of DRD2 density and binding affinity in healthy volunteers suggest that the C/C genotype is associated with the highest level of intrasynaptic dopamine in the striatum (lowest DRD2 affinity), the T/T genotype with the lowest level, and the C/T genotype with an intermediate level (Hirvonen et al., unpublished results). Given that the C957T variation of the DRD2 gene plays a significant role in striatal D2 binding, it is possible that the C957T variation could be involved in the aberrant mesolimbic and mesocortical dopamine transmission in schizophrenia. Furthermore, N-methyl-D-aspartate (NMDA) hypofunction is suggested to be associated with schizophrenia (Laruelle 2003b). It is known that dopamine D2 receptors inhibit NMDA-induced glutamate release in the substantia nigra (Marti et al. 2002) and transactivate a receptor tyrosine kinase to inhibit NMDA receptor transmission in pyramidal neurons (Kotecha et al. 2002). Increased translation and stability of DRD2 mRNA and consequent augmentation of in vivo dopamine D2 expression in subjects with the C/C genotype may result in NMDA hypofunction.

Working memory deficits are among the most pervasive cognitive symptoms of schizophrenia (Goldman-Rakic 1994), which also include decreased verbal memory and attention (Saykin et al. 1991, Cornblatt and Keilp 1994). These cognitive deficits are also observed in a milder form, in unaffected first-degree relatives of patients, suggesting a genetic contribution to these deficits (Cannon et al. 1994, Cornblatt and Keilp 1994, Park et al. 1995). In schizophrenia, there is evidence of a specific verbal memory deficit in a subgroup of patients tested on the WSPT (word serial position test) (Wexler et al. 1998, Bruder et al. 2004). In addition to positive symptoms, D2 receptors may also have a role in the cognitive symptoms. In a recent study, transgenic mice over-expressing D2 receptors selectively in the striatum were shown to have behavioural deficits in working memory without affecting spatial reference memory (Kellendonk et al. 2006). Xu et al. (2007) investigated the effect of the DRD2 C957T polymorphism on working memory in healthy individuals and found that the subjects with the C/C genotype performed poorest on the WSPT and the T/T genotype best. In addition, they found an interaction between the DRD2 C957T polymorphism and COMT (Catechol-O-Methyl-Transferase) Val158Met polymorphism (rs4680), which has also been shown to affect cognitive functions, including working memory (Egan et al. 2001, Bruder et al. 2005, Tunbridge et al. 2006).

In the first investigation of schizophrenia and the DRD2 C957T polymorphism, Lawford et al. (2005) studied 153 schizophrenic patients and 148 controls of mixed Caucasian and Northern European origin in Australia and demonstrated an association between the C allele and schizophrenia. The C/C genotype was also more frequent in schizophrenia patients than in healthy controls. The population-attributable genetic risk for this polymorphism was 24% (Lawford et al. 2005). A Spanish study of 131 schizophrenic patients and 364 healthy controls (Hoenicka et al. 2006) replicated that in the DRD C957T polymorphism the C allele and the C/C genotype were associated with schizophrenia. Also, in a Russian study (Monakhov et al. 2008), frequencies of the C957T C allele and the C/C genotype were higher in schizophrenic patients than in the control group. However, a study by Kukreti et al. (2006) with 101 schizophrenic patients and 145 controls did not reveal any association between this polymorphism and schizophrenia in South Indian subjects. Betcheva et al. (2009) performed a case-control study of 59 candidate genes in schizophrenia using 255 Bulgarian patients with schizophrenia and schizoaffective disorder and found the DRD2 C957T polymorphism as the only susceptibility factor for schizophrenia among the tested candidate genes in the Bulgarian population. Recently, Fan et al. (2010) performed a case-control association study of DRD2 gene polymorphisms with 421 schizophrenia patients and 404 healthy controls in a Chinese Han population and detected an association between the C957T polymorphism (rs6277) and schizophrenia. Contrary to most earlier studies, they found the T-allele to be a risk allele for schizophrenia (Table 5).

**Table 5.** Association studies of dopamine-2 receptor (DRD2) polymorphism and schizophrenia.

| Polymorphism | Reference | N | Result (P value, if significant) |
|---|---|---|---|
| Taq 1A | Dubertret et al. 2001 | 50 patients, 50 controls | A2 significant (P=0.004) (only in those with an onset over 20 years of age) |
| | Dubertret et al. 2004 | 103 patients, 83 controls | A2 significant (P=0.04) |
| | Lafuente et al. 2008 | 243 patients, 291 controls | Not significant |
| Ser311Cys | Itokawa et al. 1993 | 50 patients, 110 controls | Not significant |
| | Arinami et al. 1994 | 156 patients, 300 controls | Significant (P=?) |
| | Laurent et al. 1994 | 113 patients, 184 controls | Not significant |
| | Hattori et al. 1994 | 100 patients, 100 controls | Not significant |
| | Chen et al. 1996 | 114 patients, 88 controls | Not significant |
| | Tanaka et al. 1996 | 106 patients, 106 controls | Not significant |
| | Kaneshima et al. 1997 | 78 patients, 112 controls | Not significant |
| | Verga et al. 1997 | 103 patients, 97 controls | Not significant |
| | Spurlock et al. 1998 | 373 patients, 413 controls | Not significant |
| | Hori et al. 2001 | 241 patients, 201 controls | Not significant |
| | Jönsson et al. 2003 | 173 patients, 236 controls | Significant (P=0.002) (only in men) |
| | Fan et al. 2010 | 421 patients, 404 controls | Not significant |
| -141 C Ins/Del | Arinami et al. 1997 | 260 patients, 312 controls | Significant (P<0.001) |
| | Stober et al. 1998 | 260 patients, 290 controls | Not significant |
| | Ohara et al. 1998 | 170 patients, 121 controls | Significant (P=0.042) |
| | Tallerico et al. 1999 | 50 patients, 52 controls | Not significant |
| | Breen et al. 1999 | 439 patients, 437 controls | Significant (P=0.02) |
| | Jönsson et al. 1999 | 129 patients, 179 controls | Significant (P<0.05) |
| | Hori et al. 2001 | 241 patients, 201 controls | Not significant |
| | Lafuente et al. 2008 | 243 patients, 291 controls | Significant (P=0.01) |
| | Cordeiro et al. 2009 | 229 patients, 733 controls | Significant (P=0.001) |
| | Sáiz et al. 2010 | 288 patients, 421 controls | Significant (P=0.012) |
| | Kurt et al. 2011 | 73 patients, 60 controls | Not significant |
| C957T | Lawford et al. 2005 | 153 patients, 148 controls | Significant (P=0.0008) |
| | Hoenicka et al. 2006 | 131 patients, 364 controls | Significant (P=0.008) |
| | Kukreti et al. 2006 | 101 patients, 145 controls | Not significant |
| | Monakhov et al. 2008 | 311 patients, 364 controls | Significant (P=0.0435) |
| | Betcheva et al. 2009 | 255 patients, 556 controls | Significant (P=0.014) |
| | Fan et al. 2010 | 421 patients, 404 controls | Significant (P=0.034) |

Modified and updated from Lawford et al. (2005).

### 2.8.3.5 Neuregulin-1

Neuregulin-1 (NRG-1) belongs to a family of neuregulins that consists of four growth factor genes that are structurally related to the epidermal growth factor genus of cell-to-cell signalling molecules (Law et al. 2004). Neuregulins signal through erbB2, erbB3 and erbB4 receptor tyrosine kinases, which are members of the EGF receptor family (Buonanno and Fischbach 2001). The NRG-1 gene produces numer-

ous NRG-1 isoforms, all having an EGF-like domain that is sufficient for receptor binding and activation (Corfas et al. 2004). NRG-1 is localised to widespread areas in the brain, including frontal cortex, hippocampus, midbrain and cerebellum (Law 2003). NRG-1 has a broad range of bioactivities in the CNS, including synapse formation and maturation, regulation of N-methyl-D-aspartate and gamma-aminobutyric acid-A receptor subunit expression, neuron differentiation, proliferation, and migration (Anton et al. 1997, Ozaki et al. 1997, Rio et al. 1997, Rieff et al. 1999, Liu et al. 2001, Schmid et al. 2003, Krivosheya et al. 2008), as well as hormonal control of puberty (Prevot et al. 2003), regulation of acetylcholine receptors (Liu et al. 2001), and oligodendrocyte development (Canoll et al. 1996, Cannella et al. 1999, Vartanian et al. 1999, Fernandez et al. 2000, Calaora et al. 2001, Schmucker et al. 2003). These activities are partly developmental, but NRG-1 continues to be expressed in the adult brain (Law et al. 2004).

The first evidence that NRG-1-erbB signalling is altered in schizophrenia came when gene expression profiles in the prefrontal cortex of people with chronic schizophrenia and control patients were compared postmortem, which revealed a significant reduction in the level of erbB3 expression in schizophrenia (Hakak et al. 2001). This decrease was confirmed by quantitative and differential-display RT-PCR analysis (Tkachev et al. 2003). In a recent Chinese study, NRG-1 mRNA expression in peripheral blood lymphocytes (PBL) of patients was lower than that in the control groups but expression of NRG-1 mRNA of PBLs in antipsychotics-treated patients gradually increased significantly higher than it had before the therapy (Zhang et al. 2008). In addition, the atypical antipsychotic clozapine has been shown to upregulate NRG-1 in human brain aggregates (Chana et al. 2009).

The NRG-1 gene localises to chromosome 8p22. The hypothesis that defects in NRG-1-erbB signalling contribute directly to schizophrenia was strengthened by recent genetic studies linking the NRG-1 gene and the disease. A genome-wide scan of a large Icelandic sample revealed the NRG-1 gene as a susceptibility locus for schizophrenia (Stefansson et al. 2002). Stefansson et al. (2002) identified in this region an NRG-1 at-risk haplotype of seven markers and one SNP in the 5′ region of the NRG-1 gene (SNP8NRG221533, rs35753505) that was significantly different in schizophrenic patients compared to controls. The same group found associations with three SNPs and the entire haplotype in a Scottish case-control sample, providing support for the initial association (Stefansson et al. 2003). Williams et al. (2003a) examined three markers from Stefansson′s haplotype and reported an association with the haplotype but not with any of the individual markers. A study by Tang

et al. (2004) showed a strong association between NRG-1 and schizophrenia, but they found a different set of markers contributing to the at-risk haplotype. Li et al. (2004a) studied a Han Chinese population and reported a novel at-risk haplotype of the NRG-1 gene but did not confirm the Icelandic/Scottish risk haplotype. Yang et al. (2003) investigated the single most significant SNP from the study by Stefansson et al. (2002) in Chinese Han case-parent families and confirmed Stefansson´s results. On the other hand, in a Finnish case-control study, the NRG-1 SNP 221533 (rs35753505) was examined in schizophrenic patients and healthy controls, and no group differences in genotype or allele distribution were found (Kampman et al. 2004). Nor was there any association between the polymorphism and the age of onset of schizophrenia. However, genotype distributions differed between responders and non-responders to conventional antipsychotics (Kampman et al. 2004). Réthelyi et al. (2010) studied Hungarian Caucasian schizophrenic patients and found a significant association between the NRG-1 SNP 241930 marker and non-deficit schizophrenia. Petryshen et al. (2005) reported associations between a haplotype that overlaps the risk haplotype originally reported in an Icelandic population and two haplotypes located on the 3´ end of NRG-1 in a Portuguese population, but they did not detect an association with the original Icelandic risk haplotype. Turunen et al. (2007) also found suggestive evidence of an association of two SNPs in the 3´ region of the NRG-1 gene in a large Finnish family-based association analysis. Fukui et al. (2006) tested a four-SNP haplotype, which shared three SNPs of Stefansson´s original risk haplotype in a Japanese sample, and found a positive association. Zhao et al. (2004) found an association with the risk haplotype in a Chinese Han sample. In a second cohort from the Scottish population, Thomson et al. (2007) found a new region involved in schizophrenia. In a recent linkage disequilibrium-based case-control study with a Swedish population, Alaerts et al. (2009) detected an association with a 7-marker and 2-microsatellite haplotype, different from the haplotypes associated in the Icelandic population. They also found a significant association with 5 SNPs as well as with 2-, 3- and 4-SNP windows containing these SNPs (Alaerts et al. 2009). However, negative association reports have also been published (Iwata et al. 2004, Ikeda et al. 2008, Jönsson et al. 2009, Squassina et al. 2010). Thus, the results are conflicting, and no specific mutation responsible for an association has been identified to date. The most significant association is with the SNP 8NRG221533 compared with the other six markers of the at-risk haplotype (Stefansson et al. 2002, Stefansson et al. 2003, Yang et al. 2003). An additional support for the association between NRG-1 gene polymorphism and schizophrenia comes from a recent meta-analysis

in which an association with 4 SNPs and two microsatellite markers was found (Li et al. 2006). In addition, a haplotype analysis revealed an association in the pooled international populations but the risk haplotype blocks were different in Caucasian and Asian populations (Li et al. 2006) (Table 6).

**Table 6.** Association studies of neuregulin-1 (NRG-1) polymorphism and schizophrenia.

| Reference | N | Result (P value, if significant) |
|---|---|---|
| Stefansson et al. 2002 | 476 patients, 394 controls | Significant, seven-marker haplotype (P=0.000087) |
| Stefansson et al. 2003 | 609 patients, 618 controls | Significant, seven-marker haplotype (P=0.00031) |
| Williams et al. 2003 | 573 patients, 618 controls | Significant, three-marker haplotype (P=0.04) |
| Yang et al. 2003 | 246 family trios | Significant, 3 SNPs (0.00093≤P≤0.013) |
| Iwata et al. 2004 | 607 patients, 515 controls | Not significant |
| Kampman et al. 2004 | 94 patients, 395 controls | Not significant |
| Li et al. 2004a | 298 patients, 336 controls, 184 trios, 138 sibling pairs | Significant, two haplotypes (P=0.0000065 and P=0.003) |
| Tang et al. 2004 | 540 patients, 279 controls | Significant, two haplotypes (P=0.00003 and P=0.000267), four microsatellites (0.0014≤P≤0.018) |
| Zhao et al. 2004 | 369 patients, 299 controls, 352 trios | Significant, five-marker haplotype (0.0046≤P≤0.0057) |
| Petryshen et al. 2005 | 321 patients, 242 controls, 111 trios | Significant, three haplotypes (0.031≤P≤0.050) |
| Fukui et al. 2006 | 349 patients, 424 controls | Significant, four-marker haplotype (P=0.026) |
| Thomson et al. 2007 | 386 patients, 455 controls | Significant, one haplotype (P=0.024) |
| Turunen et al. 2007 | 865 patients, 441 families | Significant, two SNPs (P=0.012 and P=0.048) |
| Ikeda et al. 2008 | 2554 patients, 2526 controls | Not significant |
| Alaerts et al. 2009 | 486 patients, 514 controls | Significant, seven-marker haplotype (P=0.03), two-microsatellite haplotype (P=0.03), five SNPs (0.007≤P≤0.04) |
| Jönsson et al. 2009 | 837 patients, 1473 controls | Not significant |
| Réthelyi et al. 2010 | 280 patients, 230 controls | Significant (only in non-deficit schizophrenics), one SNP (NRG241930) (P=0.04) |
| Squassina et al. 2010 | 171 patients, 349 controls | Not significant |

NRG-1 is one of the leading candidate genes for schizophrenia (Harrison and Law 2006), but the mechanism with which risk is conferred is uncertain. There is, however, evidence that the NRG-1 polymorphism SNP8NRG243177 (rs6994992), which has been shown to be associated with risk of schizophrenia (Stefansson et al. 2003), is also associated with changes in white matter density in the right anterior internal capsule (McIntosh et al. 2008). In their study, the T allele was associated with decreased white matter intensity. In addition, the same group found, in a partially overlapping sample, that white matter integrity is reduced in the left anterior thalamic radiation in T allele carriers (Sprooten et al. 2009). Functional MRI studies have

shown aberrant frontal lobe activation in T homozygotes (Hall et al. 2006, Mechelli et al. 2008). Clinical studies suggest that in populations prone to psychosis, T allele homozygosity predicts subsequent development of psychotic illness (Hall et al. 2006, Keri et al. 2009). There is also evidence that T-allele carriers at SNP8NRG243177 have a heightened transcription rate of NRG-1 mRNA in hippocampal neurons (Law et al. 2006). Barnes et al. (2012) studied effects of the NRG-1 polymorphism SNP8NRG243177 on grey and white matter volume in 79 non-psychotic individuals from The Northern Finland 1966 Birth Cohort. They found in T allele carriers decreased grey matter in the bilateral middle and superior frontal gyrus and bilateral anterior cingulate and decreased white matter in three clusters in the frontal deep white matter and in one cluster of superior frontal white matter, suggesting that NRG-1 influences frontal lobe development both in grey and white matter. The regions of these changes are congruent with the regions implicated as abnormal in schizophrenia (Barnes et al. 2012). Taken together, these aforementioned findings suggest that NRG-1 SNP8NRG243177 has effects on structure and function of the brain that can mediate risk of schizophrenia, at least in part.

### 2.8.3.6 Other candidate genes

#### 2.8.3.6.1 Dysbindin-1 (DTNBP1)

Dysbindin-1 is a protein that is expressed neuronally (Benson et al. 2001) in postsynaptic densities in many areas of the human brain (Blake et al. 1999, Straub et al. 2002). Recent studies have demonstrated that dysbindin-1 is also located presynaptically in glutamatergic neurons and is reduced at these locations in schizophrenia (Talbot et al. 2004). Reduced expression of dysbindin-1 mRNA has been found in the dorsolateral prefrontal cortex and in certain areas of the hippocampus in schizophrenia (Weickert et al. 2004, Weickert et al. 2008). Weickert et al. (2004) also found that dysbindin-1 mRNA levels varied significantly according to the genotype at several SNPs of the dysbindin-1 gene. Dysbindin-1´s ability to bind β-dystrobrevin and, thus, interact with the dystrophin glycoprotein complex at synaptic sites implies that dysbindin-1 polymorphisms may enhance susceptibility to schizophrenia by altering synaptic mechanisms (Talbot et al. 2004). The gene-encoding dysbindin-1 is located on chromosome 6p22.3, which is one of the best-supported regions in linkage studies (Moises et al. 1995, Maziade et al. 1997, Lindholm et

al. 2001). Straub et al. (2002) picked an area within the broad 6p region linked in Irish families and used family-based association analysis of SNPs and haplotype analyses to identify the dysbindin (DTNBP1) gene. They found highly significant associations in introns 4, 5, and 6. Schwab et al. (2003a) replicated the association with one of the SNPs, and with a haplotype consisting of the three SNPs. Vilella et al. (2008) found an association of DTNBP 1 with schizophrenia at the haplotype level in intron 7. Wessman et al. (2009) reported a significant association of DTNBP 1 and a specific subtype of schizophrenia with prominent negative and positive symptoms, early age of onset, and chronic course (core schizophrenia). Recently, Réthelyi et al. (2010) have detected an association between the marker rs1011313 in DTNBP1 and non-deficit schizophrenia and they also found the two-marker haplotype composed by rs909706 and rs3213207 to be associated with schizophrenia. Additionally, two large studies have provided evidence for an association between DTNBP1 and schizophrenia (Kirov et al. 2004, Williams et al. 2004). As in other association studies, there are some studies in which no association has been found (Morris et al. 2003, Van Den Bogaert et al. 2003, Turunen et al. 2007, Jönsson et al. 2009, Strohmaier et al. 2010).

#### 2.8.3.6.2 Disrupted in schizophrenia 1 (DISC 1)

The DISC 1 gene resides on chromosome 1q42.1 (Millar et al. 2000). The carboxy-terminal region of the DISC 1 gene is disrupted by a balanced 1:11 translocation, which showed strong evidence for linkage to a fairly broad phenotype consisting of schizophrenia, bipolar disorder, and recurrent depression (Blackwood et al. 2001). In vitro studies have shown that reduced expression of endogenous DISC 1 or expression of carboxy-terminal-truncated mutant DISC 1 results in impaired neurite outgrowth (Kamiya et al. 2005). It may also lead to impaired neuronal migration and arborisation of dendritic neuronal processes of the cerebral cortex in vivo (Kamiya et al. 2005). These results indicate that loss of DISC 1 function may result in abnormal development of the cerebral cortex that, in turn, may underlie dysfunctions found in schizophrenia (Toro and Deakin 2007). DISC 1 is thought to be critical during hippocampal development and animal models have shown that the expression of DISC 1 is highest in the hippocampus (Austin et al. 2003, 2004). Because the levels of DISC 1 have been shown to be high in the neurogenic regions of the hippocampus and DISC 1 may have a role in ongoing adult neurogenesis, it has

been speculated that mutations in the DISC 1 gene may disrupt adult neurogenesis and lead to morphological and functional changes affecting the hippocampus in schizophrenia (Toro and Deakin 2007).

Linkage and association studies have supported the idea that DISC 1 is involved in the susceptibility of schizophrenia but the exact region of the gene associated with the disorder is still unclear (Ekelund et al. 2001, Hodgkinson et al. 2004, Callicott et al. 2005, Zhang et al. 2006). However, in a recent association study, Schumacher et al. (2009) identified in a Central European sample a risk interval in DISC 1 intron 9 and they also performed a meta-analysis across different European populations showing a risk interval in DISC 1 introns 4–6. An SNP of the DISC 1 gene (Ser704Cys, rs821616) has been found to be associated with reduced hippocampal volume by MRI and abnormal BOLD fMRI activity during short-term and long-term memory tasks in the normal population (Callicott et al. 2005). Lepagnol-Bestel et al. (2010) analysed four SNPs of the DISC 1 gene in a sample of 214 parent-offspring schizophrenia trios of French and Algerian origin and found a significant association with the SNP C1872T (rs6675281), locating very close to the translocation breakpoint. There are also negative results with the DISC 1 gene. Hotta et al. (2011) performed a case-control association study of four candidate SNPs with 485 Japanese schizophrenia patients and 660 healthy controls and found no association with schizophrenia.

#### 2.8.3.6.3 Catechol-O-methyltransferase (COMT)

The Catechol-O-methyltransferase (COMT) gene is located on chromosome 22q11, a region that has been linked to schizophrenia in several linkage studies (Lewis et al. 2003). COMT degrades catecholamines with a particular role in cortical dopamine metabolism (Gogos et al. 1998). As aberrations in dopaminergic transmission have long been implicated in schizophrenia, COMT is a candidate gene in its own right. In addition, in velocardiofacial syndrome (VCFS) there is both a deletion of the 22q11 region and a heightened risk of schizophrenia (Murphy 2002). Egan et al. (2001) investigated the codon 158 Val/Met polymorphism (rs4680) in the COMT gene and found that this polymorphism slightly increased risk of schizophrenia. Shifman et al. (2002) found in a large Israeli Ashkenazi Jewish population significant associations between schizophrenia and SNPs in the gene for COMT. Hoenicka et al. (2010) detected a significant association between the Val158Met polymorphism

and schizophrenia in a Spanish male population, but regarding females they found no statistically significant association between the COMT SNP and schizophrenia. Wan et al. (2011) performed a case-control association study of the Val158Met genetic polymorphism in a Malaysian sample of 317 schizophrenia patients and 417 healthy controls and observed a weak association with the disease. Contrary to Hoenicka et al.′s study (2010), Wan et al. (2011) found the association only in females. There are also negative studies concerning the association between the COMT gene polymorphism and schizophrenia (Williams et al. 2005, Zhang et al. 2012). Furthermore, a meta-analysis by Lohmueller et al. (2003) did not support an association between the COMT gene and schizophrenia.

#### 2.8.3.6.4 D-amino acid oxidase (DAAO) and D-amino acid oxidase activator (DAOA)

The gene for D-amino acid oxidase (DAAO/DAO) is located on chromosome 12q24 and the gene for D-amino acid oxidase activator (DAOA, also referred to as G72) is located on chromosome 13q34, which is an area that has been linked to schizophrenia in previous studies (Badner and Gershon 2002, Owen et al. 2004). These genes were first discovered by Chumakov et al. (2002) who found a significant association between schizophrenia risk and an area called Bin A in which they identified two genes: G72 (DAOA) and G30. It was first thought that the DAOA protein acts as an activator of the DAAO protein (Chumakov et al. 2002), but more recent data suggest that DAOA regulates mitochondrial function without interacting with DAAO (Kvajo et al. 2008). DAAO plays an important role in the metabolism of D-amino acids such as D-serine. D-serine is a co-agonist of the glutamate receptor which, in turn, is implicated in the pathogenesis of schizophrenia. Growing evidence suggests that glutamate hypofunction is associated with symptoms of schizophrenia (Tuominen et al. 2005). Later, the association between DAOA and DAAO and schizophrenia has been confirmed in several studies (Korostishevsky et al. 2004, Schumacher et al. 2004, Wang et al. 2004, Hong et al. 2006, Ma et al. 2006, Yue et al. 2006, Opgen-Rhein et al. 2008), although negative results also exist (Mulle et al. 2005, Ohi et al. 2009). Wood et al. (2007) found a positive association between DAO and schizophrenia but not between DAOA and schizophrenia. In a recent longitudinal study with two MRI scans performed an average of 3 years apart, a four-SNP haplotype of the DAOA gene was associated with an increased rate of

frontal lobe tissue loss in schizophrenic patients (Hartz et al. 2009). In addition, this same haplotype was also associated with more severe psychotic symptoms at the time of the second MRI scan (Hartz et al. 2009).

#### 2.8.3.6.5 Regulator of G-protein signalling-4 (RGS-4)

The RGS-4 gene maps to the putative linkage region on chromosome 1q22. RGS proteins function primarily as GTPase-activating proteins (GAPs) for heterotrimeric G-protein α (Gα) subunits, accelerating the hydrolysis of Gα-bound GTP (DeVries et al. 2000). Thus, they shorten the duration of intracellular signalling of many G-protein-coupled receptors (GPCRs) belonging to dopamine, GABA, glutamate and other neurotransmitter systems (Chowdari et al. 2002). RGS-4 was targeted for genetic analysis following a microarray-based gene-expression study which showed decreased RGS-4 expression in a schizophrenic post-mortem brain (Kirov et al. 2005). Chowdari et al. (2002) analysed 26 SNPs in the RGS-4 gene, and several in the upstream sequence and first intron were associated with schizophrenia. Evidence of an association was seen in three family samples, although the pattern varied between them. Thereafter, several studies have confirmed this association between the RGS-4 gene and schizophrenia (Chen et al. 2004, Morris et al. 2004, Williams et al. 2004, Zhang et al. 2005), while others have not (Cordeiro et al. 2005, Guo et al. 2006, Kampman et al. 2006, Rizig et al. 2006, Ishiguro et al. 2007, Réthelyi et al. 2010, Jönsson et al. 2012).

#### 2.8.3.6.6 Proline dehydrogenase (PRODH)

The proline dehydrogenase gene is located at the VCFS region on chromosome 22q11. Linkage disequilibrium mapping of the region in patients identified a segment containing the proline dehydrogenase gene as a candidate for schizophrenia (Liu et al. 2002). They also analysed SNPs and haplotypes of the PRODH gene and found an association between this gene and schizophrenia in adult- and early-onset patients. Li et al. (2004b) studied six SNPs in the PRODH gene and found a haplotype that was associated with schizophrenia. Kempf et al. (2008) showed in a family-based sample an association between functional polymorphisms of the PRODH gene and schizophrenia. The results concerning the PRODH gene are,

however, clearly contradictory because there are also several negative reports (Fan et al. 2003, Williams et al. 2003b, Ohtsuki et al. 2004, Abou Jamra et al. 2005, Glaser et al. 2006).

### 2.8.4 Genome-wide association studies and copy number variation

Genome-wide association (GWA) studies use dense maps of SNPs that cover the human genome to look for allele-frequency differences between cases and controls (Kruglyak 2008). Most of the genomic DNA sequence differences between any two people are common (frequency >5%) SNPs. Because of localised patterns of correlation (linkage disequilibrium), 500,000 to 1,000,000 of these SNPs are sufficient to provide evidence that one or more common variants explain part of the genetic risk of a disease ("common disease, common variant" hypothesis) (Psychiatric GWAS Consortium Coordinating Committee 2009). At the time, currently available GWAS platforms containing about 1,000,000 SNPs adequately cover over 90% of the genome for those of European or East Asian ancestry (Psychiatric GWAS Consortium Steering Committee 2009). The genome-wide association approach permits studying the entire human genome at levels of resolution previously unattainable, in very large study populations of thousands of unrelated individuals. Copy number variants are chromosomal segments where DNA has been deleted or duplicated. Copy number variation (CNV) means submicroscopic cytogenetic variation including genomic deletions and duplications of more than 1 kilobase pair (kb) in size (Buizer-Voskamp et al. 2011). CNV is an important source of sequence variation between individuals and it has been postulated that it could contribute to phenotypic variation (O´Donovan et al. 2009). GWAS technologies can also detect some of the copy number variants in the genome (Psychiatric GWAS Consortium Coordinating Committee 2009).

In the recent few years, several GWA studies have been performed in psychiatric disorders. Concerning schizophrenia, the first published GWA study was based upon only 178 cases and 144 controls (Lencz et al. 2007). They reported only their strongest association ($P= 3.7 \times 10^{-7}$), which was to a marker between colony-stimulating factor 2 receptor alpha and short stature homeobox isoform b, each gene being about 350 kb from the associated marker. O´Donovan et al. (2008) performed initial GWAs on 479 cases and 2937 controls, and then followed up loci surpassing $P < 10^{-5}$ in up to 6829 cases and 9897 controls. The strongest association was in an intron of

zinc finger protein 804A (ZNF804A) on chromosome 2q32.1. The association in schizophrenia was not significantly genome-wide ($P = 1.61 \times 10^{-7}$), but when the patients with bipolar disorder were also included, the threshold was surpassed ($P = 9.96 \times 10^{-9}$). Therefore, ZNF804A is considered a very likely susceptibility locus for psychosis, although the risk increment is small (OR about 1.09) (O´Donovan et al. 2008). Since then, subsequent GWAS (International Schizophrenia Consortium 2009) and case-control association studies (Riley et al. 2010, Steinberg et al. 2011a) have replicated the results and highlighted ZNF804A as a strong genome-wide supported susceptibility gene for schizophrenia. In a recent study, ZNF804A was shown to regulate transcriptionally the expression of four schizophrenia-associated genes (COMT, DRD2, PRSS16 (protease, serine, 16 [thymus]), PDE4B (phosphodiesterase 4B, cAMP-specific)) in rat cortical progenitor cells (Girgenti et al. 2012).

Stefansson et al. (2009) performed a genome-wide scan of 2663 schizophrenia patients and 13,498 controls from eight European locations and analysed 314,868 SNPs for a potential association with schizophrenia (collectively called SGENE-plus). Next, they combined findings from their top 1500 markers with results for the same markers from both the International Schizophrenia Consortium (ISC) (2602 cases and 2885 controls, overlapping samples excluded) and the European-American portion of the Molecular Genetics of Schizophrenia (MGS) (2681 cases and 2653 controls). Twenty-five of their 1500 top markers had P values less than $1 \times 10^{-5}$ in the combined results. These top markers were followed up in 4999 cases and 15,555 controls from four sets of additional samples from Europe. A significant association with five markers spanning the major histocompatibility complex (MHC) region on chromosome 6p21.3-22.1 was found, which is consistent with earlier investigations suggesting immune system involvement in schizophrenia. In addition, they reported a significant genome-wide association with a marker located upstream of the neurogranin gene on 11q24.2 and a marker in intron four of transcription factor 4 on 18q21.2. These two latter observations refer more to perturbation of pathways involved in brain development and cognitive function, especially memory (Stefansson et al. 2009). The association with 6p22.1 was also supported by a meta-analysis of European-ancestry subjects by Shi et al. (2009). Later, Stefansson´s group extended their earlier study by examining loci having $P < 1 \times 10^{-4}$ in the SGENE-plus-ISC-MGS data set (Steinberg et al. 2011b). They tested all SNPs in 19 European and European-American study groups, which consisted of 9246 cases and 22,356 controls. The SNPs reaching genome-wide significance in a joint analysis (SGENE-plus-ISC-MGS and follow-up) were studied in an additional 1014 cases

and 1144 controls from Germany. They found two new variants — one at 2p15.1 and the other at 18q21.2 — showing genome-wide association with schizophrenia. The novel variant at 2p15.1, rs 2312147, is located about 50 kb upstream of VRK2, a gene coding for a serine/threonine kinase. The other newly identified risk variant at 18q21.2, rs 4309482, is situated approximately equidistant from CCDC68 and TCF4, perhaps acting through either the gene or both. Additionally, they found six other variants showing significantly genome-wide associations in the MHC region, at 11q24.2, and at 18q21.2 (Steinberg et al. 2011b).

The International Schizophrenia Consortium (ISC) (2009) conducted a genome-wide association study of 3322 European patients with schizophrenia and 3587 controls. The most associated genotyped SNP ($P=3.4\times10^{-7}$) was found from the first intron of myosin XVIIIB (MYO18B) on chromosome 22. The second strongest association consisted of more than 450 SNPs on chromosome 6p spanning the major histocompatibility complex (MHC). They also found associations in the 22q11.2 deletion region and ZNF804A. The second approach of the International Schizophrenia Consortium (2009) was to test the theory of polygenic inheritance in schizophrenia. Therefore, they summarised variation across nominally associated loci in quantitative scores, and related the scores to the disease state in independent samples. For the analysis, 74,062 autosomal SNPs in approximate linkage disequilibrium were chosen. The results showed that score alleles were significantly enriched among target cases ($P=9\times10^{-19}$), explaining ~3% of the variance. The results were not driven by only a few highly associated regions. To replicate the polygenic component, they used independent GWAS samples, of which two European schizophrenia samples showed ISC-derived scores that were highly associated with disease ($P=2\times10^{-28}$ and $P=5\times10^{-11}$). Also, in an African-American sample, cases carried more of the European-derived score alleles than their African-American controls (P=0.008). The ISC-derived score alleles also proved to be associated with bipolar disorder in two independent samples ($P=7\times10^{-9}$ and $P=1\times10^{-12}$), indicating a prominent shared genetic component. They conclude that their molecular genetic data strongly support a polygenic basis to schizophrenia involving common SNPs and explaining at least one third of the total variation in liability, but do not exclude contributions of rare variants for schizophrenia.

Athanasiu et al. (2010) performed a GWA study of schizophrenia using a two-step study design consisting firstly of a smaller sample as a hypothesis-generating step (discovery sample) and then secondly a larger one as a hypothesis-verification step to replicate the findings and separate potentially true associations from false

positives (replication sample). The discovery sample consisted of 201 cases and 305 healthy controls, which were ethnically homogenous Norwegian subjects. The subjects in the replication analysis consisted of 2663 cases and 13,780 controls, which were recruited from several European countries. In the discovery sample, no SNP reached the corrected P value of $8.7 \times 10^{-8}$ corresponding to $P < 0.05$ after adjusting for 572,888 independent tests, which were performed in this phase. However, the analysis revealed seven loci, represented by 11 SNPs that were moderately associated with schizophrenia ($P < 1 \times 10^{-5}$). For the replication phase, the 1000 strongest signals in the discovery sample were chosen to test the potential association with schizophrenia and to separate potentially true associations from false negatives. Then, a combined analysis of both samples was performed. This analysis revealed 32 nominally significant associations with schizophrenia ($P < 1 \times 10^{-5}$) with odds ratios between 1.1 and 1.2. The strongest signals were found with markers in genes encoding the phospholipase A2-activating protein, acyl-CoA synthetase medium-chain family member 1 and ankyrin 3, node of Ranvier (ANK 3). Interestingly, the ANK 3 gene has also been shown to associate with bipolar disorder, supporting a common etiology of these two disorders (Lichtenstein et al. 2009, Schultze et al. 2009). It is also remarkable that Athanasiu et al. (2010) reported some previously identified candidate genes — DISC1, RELN, NRG1, and OPCML — nominally significantly associated with schizophrenia in the discovery sample.

The Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium (2011) recently conducted a large genome-wide association study of schizophrenia. It was a two-stage study in which there were in the first discovery stage a sample of 21,856 individuals of European ancestry and in the second replication sample 29,839 independent subjects. In the first stage, a mega-analysis combining GWAS data from 17 separate studies (with a total of 9394 cases and 12,462 controls) was conducted. In this stage, 136 associations reached genome-wide significance ($P < 5 \times 10^{-8}$). The majority of these associations (N=129) mapped to the extended major histocompatibility complex (MHC, 6p21.32-p22.1), a region already previously implicated in schizophrenia. The other stage 1 regions included new regions (10q24,33 and 8q21.3) and previously reported regions (18q21.2 and 11q24.2). In stage 2, the most significant SNPs (N=81) in each LD region where at least one SNP had surpassed $P < 2 \times 10^{-5}$ in the mega-analysis were evaluated in 29,839 independent subjects (8442 cases and 21,397 controls). In the MHC region, five of 22 SNPs surpassed the threshold of being significantly genome-wide in stages 1 and 2 combined, with minimum $P=2.2 \times 10^{-12}$. In the combined dataset

(stages 1 and 2), five new (1p21.3, 2q32.3, 8p23.2, 8q21.3 and 10q24.32-q24.33) and two previously reported (6p21.32-p22.1 and 18q21.2) loci met genome-wide significance. The strongest new association was at 1p21.3 (rs1625579; $P=1.6\times10^{-11}$), which is within intron 3 of AK094607 containing the primary transcript for microRNA 137 (MIR137). MIR137 has been suggested to have a role in regulating adult neurogenesis (Silber et al. 2008) and neuronal maturation (Smrt et al. 2010); therefore, its genetic variation could possibly contribute to brain development abnormalities in schizophrenia.

Yue et al. (2011) performed a two-stage genome-wide association study of schizophrenia in the Han Chinese population with 746 schizophrenic patients and 1599 healthy controls in the first stage, and 4027 schizophrenic patients and 5603 healthy controls in an independent cohort of Han Chinese individuals in the replication analysis. The replication study was conducted by genotyping 46 promising SNPs in 38 loci ($P_{GWAS}<1\times10^{-5}$) found in the discovery stage further in the replication sample. They found six SNPs located in the 6p21-p22 and 11p11.2 loci that provided independent, consistent evidence of an association in the replication group and highly significant association in the combined studies reaching genome-wide significance (combined P values: 6p21-p22: rs1233710, $P=4.76\times10^{-11}$; rs 1635, $P=6.91\times10^{-12}$; rs2142731, $P=5.14\times10^{-10}$ and 11p11.2: rs11038167, $P=1.09\times10^{-11}$; rs11038172, $P=7.21\times10^{-10}$; rs835784, $P=2.73\times10^{-11}$). The three validated SNPs within 6p21-p22.1 were located in the extended MHC area, which has been reported to be associated with schizophrenia by previous studies (International Schizophrenia Consortium 2009). At 6p21-p22.1, Yue et al. (2011) identified three genes, including NKAPL (encoding NFKB-activating protein-like), ZKSCAN4 (encoding zinc finger with KRAB and SCAN domains 4) and PGBD1 (encoding PiggyBac transposable element-derived 1), and at the 11p11.2 TSPAN18 (tetraspanin 18) gene. The strongest association was with the SNP rs1635 near the NKAPL gene, which is suggested to have a role in the regulation of neuronal migration during early neurodevelopment (Yue et al. 2011).

Shi et al. (2011) conducted a GWAS study in the Han Chinese population in two stages to identify new common genetic risk factors in schizophrenia. Firstly, they analysed 546,561 SNPs in 3750 individuals with schizophrenia and 6468 healthy controls and then continued their study by analysing the strongest association signals in an additional independent cohort of 4383 cases and 4539 controls. In the combined analysis, two SNPs in 8p12 (rs16887244 and rs1488935) and one SNP in 1q24.2 (rs10489202) reached genome-wide significance ($P < 5.0 \times 10^{-8}$). The

rs1688935 SNP is located in intron 1 of LSM1 and in the expression data from the brain, it is nominally associated with the expression of ASH2L, DDHD2, PPAPDC1B and LETM2 genes ($P < 0.05$). The rs 1488935 SNP is located ~135 kb upstream of FGFR1 encoding the fibroblast growth factor receptor 1 protein. This is notable because there is growing evidence that fibroblast growth factors (FGF) can have a role in schizophrenia, including positional and functional genetic studies, knockout mouse models and examination of the effects of FGF in animals and humans (Shi et al. 2011). The rs10489202 is located in intron 1 of BRP44, which encodes brain protein 44. Notably, rs 10489202 is located ~140 kb downstream of MPZL1 encoding the myelin protein zero-like 1, which has been shown to be significantly upregulated in schizophrenia patients relative to healthy controls (Shi et al. 2011).

Betcheva et al. (2013) performed a two-stage GWAS study of schizophrenia in a small Bulgarian sample using firstly 188-patient and 376-control samples for the GWAS and 99-patient and 328-control samples for the conformation analysis. Firstly, in the GWAS part of their study, 495,089 SNPs were analysed, and for the replication genotyping, one hundred SNPs showing the smallest P values were regenotyped. The combined analysis of the first and second parts of the study revealed an SNP rs 7527939 showing a possible association with schizophrenia with a P value of $6.49 \times 10^{-9}$. The significance level after Bonferroni correction was set at $1.0 \times 10^{-7}$ (0.05/495089). rs 7527939 is located within intron 2 of the HHAT (hedgehog acyltransferase) on chromosome 1q32.2. HHAT controls the activity of SSH (Sonic hedgehog), a secreted glycoprotein that plays a critical role in ventral neural tube formation and axon guidance. SSH has an important role in inducing differentiation of the dopaminergic neuroblasts and cell migration towards specific sites of dopaminergic neurons in the mammalian brain (Betcheva et al. 2013). The authors hypothesise that the altered activity of the SSH gene, regulated by the genetic variations of the HHAT gene, could cause errors in neuronal cell migration and interaction during embryonic development to have an impact on schizophrenia susceptibility (Betcheva et al. 2013) (Table 7).

**Table 7.** Genome-wide association (GWA) studies in schizophrenia.

| Reference | Major findings (P value) |
|---|---|
| Lencz et al. 2007 | Association with a marker between colony-stimulating factor 2 receptor alpha and short stature homeobox isoform b ($P=3.7\times10^{-7}$) |
| O´Donovan et al. 2008 | Association with zinc finger protein 804A (ZNF804A) ($P=9.96\times10^{-9}$, together with bipolar disorder) |
| Stefansson et al. 2009 | Association with five markers in the major histocompatibility complex (MHC) ($1.4\times10^{-12}\leq P\leq1.1\times10^{-9}$)<br>Association with a marker located upstream of the neurogranin gene on 11q24.2 ($P=2.4\times10^{-9}$)<br>Association with a marker in intron four of transcription factor 4 on 18q21.2 ($P=4.1\times10^{-9}$) |
| Shi et al. 2009 | Association with seven SNPs in the extended MHC region on 6p22.1 ($9.54\times10^{-9}\leq P\leq3.81\times10^{-8}$) |
| International Schizophrenia Consortium 2009 | Association with an SNP in the first intron of myosin XVIIIB (MYO18B) ($P=3.4\times10^{-7}$)<br>Association with more than 450 SNPs on chromosome 6p spanning the MHC<br>Evidence of substantial polygenic component to the risk of schizophrenia (thousands of common alleles of very small effect) |
| Athanasiu et al. 2010 | Association with a marker (SNP) in gene-encoding phospholipase A2-activating protein (PIAA) ($P=2.12\times10^{-6}$)<br>Association with a marker (SNP) in gene-encoding acyl-CoA synthetase medium-chain family member 1 (ACSM1) ($P=3.27\times10^{-6}$)<br>Association with a marker (SNP) in gene-encoding protein ankyrin 3, node of Ranvier (ANK3) ($P=7.68\times10^{-6}$) |
| Ikeda et al. 2011 | No significant genome-wide associations. Strongest associations: ornithine aminotransferase (OAT) ($P=6.2\times10^{-6}$), sulfotransferase family, cytosolic, 6B, member 1 (SULT6B1) ($P=8.0\times10^{-6}$) |
| The Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium 2011 | Significant genome-wide association for seven loci. Strongest associations: MHC region 5 SNPs (minimum $P=2.2\times10^{-12}$), microRNA 137 (MIR137) ($P=1.6\times10^{-11}$), and coiled-coil domain containing 68 (CCDC68) ($P=2.6\times10^{-10}$) |
| Steinberg et al. 2011b | Association with a marker (SNP) locating upstream of the vaccinia-related kinase 2 (VRK2) gene on 2p15.1 ($P=1.9\times10^{-9}$)<br>Association with a marker (SNP) locating equidistant from the coiled-coil domain containing 68 (CCDC68) gene and the transcription factor 4 (TCF4) gene on 18q21.2 ($P=7.8\times10^{-9}$)<br>Association with six variants in the MHC region, at 11q24.2 and 18q21.2 ($1.4\times10^{-13}\leq P\leq4.2\times10^{-9}$) |
| Yue et al. 2011 | Association with three markers (SNPs) at 6p21-p22.1 ($6.91\times10^{-12}\leq P\leq5.14\times10^{-10}$)<br>Association with three markers (SNPs) at 11p11.2 ($1.09\times10^{-11}\leq P\leq7.21\times10^{-10}$) |
| Shi et al. 2011 | Association with two markers (SNPs) at 8p12, one locating in intron 1 of LSM1 and the other near FGFR1 ($1.27\times10^{-10}\leq P\leq5.06\times10^{-9}$)<br>Association with a marker (SNP) at 1q24.2 locating in intron 1 of BRP44, encoding brain protein 44 ($P=9.50\times10^{-9}$) |
| Betcheva et al. 2013 | Association with a marker (SNP) rs 7527939 located within intron 2 of the HHAT (hedgehog acyltransferase) on chromosome 1q32.2 ($P=6.49\times10^{-9}$) |

Studies of copy number variants (CNV) in schizophrenia have shown significant differences between cases and controls in the number of CNVs, and several genes disrupted by CNVs in schizophrenia have been proposed (Merikangas et al. 2009). Recent studies have implicated rare, large, high-penetrance copy number variants in some cases (Cook and Scherer 2008), but the genes or biological mechanisms that underlie susceptibility are not known. There is, however, evidence that schizophrenia can be caused by rare mutations in genes in pathways of neuronal development and regulation (Walsh et al. 2008). Stefansson et al. (2008) performed a genome-wide search for *de novo* CNVs associating with schizophrenia and found three deletions at 1q21.1, 15q11.2 and 15q13.3 associating significantly with schizophrenia in their sample of 1433 schizophrenia patients and 33,250 controls. In phase II they studied a sample of 3285 patients and 7951 controls and also the three deletions associated significantly with schizophrenia in the combined group. The International Schizophrenia Consortium (2008) reported a genome-wide study of rare CNVs in 3391 schizophrenia patients and 3181 controls. They found an association between schizophrenia and deletions within the region 22q11.2, which is the critical region for velo-cardio-facial syndrome with 30% risk for psychotic symptoms. They also detected an association between schizophrenia and large deletions on chromosomes 15q13.3 and 1q21.1.

McCarthy et al. (2009) studied the possible association between schizophrenia and recurrent microdeletions and microduplications of a 600-kb genomic region of chromosome 16p11.2 in two large independent cohorts, with the initial cohort containing 1906 patients and 3971 controls and the replication cohort 2645 patients and 2420 controls. They found that the 16p11.2 microduplication was associated with a 14.5-fold increased risk of schizophrenia in the combined sample. They also performed a meta-analysis of data on schizophrenia, bipolar disorder and childhood developmental disorders by integrating the data from their study into four previously publicly available datasets, which consisted totally of 8590 schizophrenia patients, 2172 patients with developmental delay or autism, 4822 bipolar patients and 30,492 controls. In this combined sample, the microduplication of 16p11.2 was strongly associated with schizophrenia (OR=8.4) and autism (OR=20.7), and also significantly associated with bipolar disorder (OR=4.3). The reciprocal microdeletion was strongly associated with developmental delay or autism (OR=38.7), but neither with schizophrenia nor with bipolar disorder. The 16p11.2 microduplication contains 28 genes, including several genes with potential roles in neurodevelopment, and at least 17 of them are expressed in the mammalian brain (McCarthy et al. 2009).

Gene knock-out models of mice have shown behavioural features with several of these duplicated genes, but it is not known which genes in this region contribute to increased risk of psychiatric and neurodevelopmental disorders (McCarthy et al. 2009).

Magri et al. (2010) performed a genome-wide screening for copy number variations in an Italian sample of 180 patients with schizophrenia and 171 healthy controls. They found five patients with a CNV occurring in one of the regions most impressively implicated as risk factors of schizophrenia: neurexin-1 (NRXN1) and the 16p13.1 regions were found to be deleted in single patients and 15q11.2 in two patients, whereas the 15q13.3 region was duplicated in one patient. Furthermore, they detected five large CNVs (>900 kb) in 4q32, 5q14.3, 8q23.3, 11q25 and 17q12 in five different patients possibly including some new candidate schizophrenia susceptibility genes. The results of Magri et al.´s (2010) study further support the proposed model of schizophrenia that includes the effect of multiple rare, highly penetrant variants, but contrary to some earlier reports, they did not find a generalised increase in all of the CNVs combined.

Buitzer-Voskamp et al. (2011) performed a systematic genome-wide CNV analysis within a cohort of 834 Dutch schizophrenia patients and 672 unaffected Dutch control individuals. They found significantly more deletions, but not duplications, in schizophrenia cases than in control subjects, suggesting that chromosomal deletions could be a risk factor for pathogenicity. The CNVs identified confirm earlier findings of the following cytogenetic regions of interest: 1q42, 2p25, 15q13, and 22q11, as well as a potentially new region of interest on chromosome 5q35.

Levinson et al. (2011) analysed rare CNVs of 3945 subjects of schizophrenia or schizoaffective disorder and 3611 screened comparison subjects who were either of European ancestry or were African Americans. Their study confirmed the previously reported associations between schizophrenia and 1q21.1, 15q13.3, and 22q11.31 deletions, 16p11.2 duplications, and exonic NRXN1 deletions. They also found a new candidate CNV, a 1.6-Mb deletion, in 3q29, which has formerly been reported to cause mild-moderate mental retardation, microcephaly, autism, and inconsistent physical anomalies (Ballif et al. 2008). In addition, Levinson et al. (2011) detected a candidate association between exonic duplications of the gene for vasoactive intestinal peptide receptor 2 (VIPR2) and schizophrenia. Their study showed in the case subjects a modestly higher genome-wide number of gene-containing deletions, but not duplications.

Grozeva et al. (2012) wanted to study the robustness and specificity of the former CNV findings in schizophrenia by analysing these loci in a large population of 10,259 individuals from the UK Wellcome Trust Case Control Consortium (WTCCC) who were affected by six non-psychiatric disorders (coronary artery disease, Crohn´s disease, hypertension, rheumatoid arthritis, types 1 and 2 diabetes) and comparing them with the previous findings with schizophrenia patients. They chose loci that had received replicated evidence from several large studies and compared the frequency of CNVs in the WTCCC reference set with that in the schizophrenia cases taken from the largest published studies or reviews (for 1q21.1, 15q13.3, 16p11.2, 17p12, 17q12 and 22q11.2) or from combined data from studies that had at least 1000 patients and 1000 controls (for 3q29, 15q11.2, and 16p13.1) (Grozeva et al. 2012). Their results show that six of these loci remain significantly more common in schizophrenia patients than in the WTCCC reference population (1q21.1, 3q29, 15q11.2, 15q13.1, 16p11.2, 22q11.2), providing strong support that these CNVs are real risk factors for schizophrenia. The differences with the three remaining loci (16p13.1, 17p12 and 17q12) were not statistically significant (Grozeva et al. 2012).

Recently, Ye et al. (2012) have published the first analysis of copy number variations in brain DNA from patients with schizophrenia and other psychiatric disorders. All former CNV studies have been performed on peripheral cells or transformed cell lines, which replicate repeatedly throughout life. Because it is possible that CNVs can be generated during cell division it is important to confirm that such anomalies are found in brain DNA in association with clinical illness. Their aim was also to show that these structural anomalies impact on expression of genes in the affected regions, because their pathogenicity is presumably related to such effects. Their study sample consisted of 600 brains, including 441 individuals with various psychiatric diagnoses. Among them were 193 patients with a diagnosis of schizophrenia. The DNA was extracted from cerebellar tissue and RNA for the expression study was extracted from the dorsolateral prefrontal cortex (DLPFC). They found 10 patients with schizophrenia or autism that had a CNV in a region that has previously been identified as associated with schizophrenia. The CNVs were found in four schizophrenia patients and they consisted of deletions in regions, 22q11, associated with velo-cardio-facial syndrome, 1q21.1 and 11q25, and a duplication in 15q11.2. Ye et al. (2012) assessed the expression profiles of genes within the CNVs and compared seven of the 10 cases to control subjects with a whole-genome approach. In a schizophrenia patient with a deletion in the 22q11 region, COMT was dramatically downregulated and 19 other hemi-deleted genes showed very low

levels of expression in comparison with other subjects, whereas a slight increase in COMT expression was seen in a depression patient with the 22q11 duplication. As a whole, gene expression in the deletion cases tended to be rather low but not as distinctly extreme as might have been expected. Ye et al. (2012) speculate that this could be due to the ability of the intact chromosome to compensate the lowered gene expression. Gene expression of the duplication cases did not differ consistently from the other samples in this study. The amount of CNVs in regions previously associated with schizophrenia was in this study (4/193) at the expected level (2–3%); however, no overall greater gene-centric CNV burden across the genome in schizophrenia patients compared to the control population could be shown. This finding differs from some earlier studies in which a very small increase in the genome-wide CNV burden has been reported. According to Ye et al. (2012), their study can be underpowered to make similar observations.

### 2.8.5 Velo-cardio-facial syndrome and schizophrenia

As stated before, velo-cardio-facial syndrome (VCFS) with a chromosome 22q11.2 deletion has been associated with schizophrenia (International Schizophrenia Consortium 2008), and it is considered one of the major recurrent CNVs associated with schizophrenia (Tam et al. 2009). The VCFS phenotype is complex, including mild facial dysmorphic features, learning difficulties, hypernasal speech, and congenital abnormalities such as congenital heart defects and velopharyngeal insufficiency (Bassett and Chow 2008). VCFS is caused by the recurrent deletion of a 3-megabase (mB) region containing more than 45 genes or sometimes a smaller 1.5-mB region (Bassett and Chow 2008, Tam et al. 2009). The deletion is most commonly a spontaneous (de novo) mutation that occurs during gametogenesis (Bassett and Chow 2008). The prevalence of VCFS is unknown and is likely to vary according to the population´s demographic characteristics (Bassett and Chow 2008). The best available estimates based largely on infants with congenital anomalies leading to genetic testing refer to the prevalence of VCFS of about 13 in 100,000 (Goodship et al. 1998). VCFS strongly predisposes to behavioural abnormalities, such as schizophrenia, bipolar disorder, autism, and other psychoses, with a prevalence of about 30% (Tam et al. 2009). On the other hand, studies suggest that the overall prevalence of 22q11.2 deletions in schizophrenia is 0.9–1% (Bassett et al. 2010), but higher estimates up to 2% in adult-onset schizophrenia (Wiehahn et al. 2004) and 4% in

childhood-onset cases (Sporn et al. 2004) have also been obtained. Several studies (but not all) have shown that the major clinical features of VFCS schizophrenia are largely indistinguishable from other forms of schizophrenia (Gothelf et al. 1999, Bassett et al. 2003, Sporn et al. 2004). However, there can be some differences in auxiliary clinical features, such as lower rates of comorbid substance use disorders and greater severity of excitement and impulsivity (Bassett et al. 2003).

The 22q11 deletion is considered a true subtype of schizophrenia (Tam et al. 2009). It has been suggested that the increased risk of schizophrenia probably results from haploinsufficiency of one or more genes or unmasking of harmful genetic variants at 22q11, and several functional candidates within the deleted region have been proposed, including COMT, PRODH, the micro-RNA processing gene DiGeorge syndrome critical region 8 (DGCR8), and the brain-expressed putative palmitoyltransferase ZDHHC8 (Tam et al. 2009). Despite this, the exact genes predisposing to schizophrenia are still unknown (Tam et al. 2009).

## 2.9 Gene-environment interaction and epigenetics in schizophrenia

Studies suggest that there might be gene-environment interaction in the etiology of schizophrenia. It has been shown that risk of schizophrenia spectrum disorder or schizophrenia thought disorder is higher in high-risk adoptees that are brought up in a dysfunctional adoptive family environment (Tienari et al. 1994, Wahlberg et al. 1997). In a recent population study the risk of schizophrenia was found to be significantly higher in the offspring with both maternal depressed mood during pregnancy and parental psychosis than in those with a depressed mother but without parental psychosis (Mäki et al. 2010). In this study, familial risk of psychosis was considered a genetic risk factor and mothers´ depressed mood an environmental or genetic risk factor. There are also a few reports which have examined the relationship between an environmental risk factor and a particular variant of a genetic polymorphism. Narita et al. (2000) found a significantly higher proportion of births during February and March among schizophrenic patients presenting the human leukocyte antigen HLA-DR1. Tochigi et al. (2002) did not find any association between season of birth and HLA-A variants in schizophrenic patients. Chotai et al. (2003) studied the relationship between three gene polymorphisms (tryptophan hydroxylase (TPH), serotonin transporter (5-HTTLPR) and dopamine receptor

(DRD4)) and the season of birth and found significant season of birth variations in three candidate genes studied. In addition, in a birth cohort followed to adulthood, a functional polymorphism in the COMT gene interacted with adolescent-onset cannabis use to predict the emergence of adult psychosis (Caspi et al. 2005).

One problem in our understanding about gene-environment interplay is that current findings are purely statistical in nature; statistical evidence of an interaction reveals little about the molecular pathologic mechanisms underlying psychosis (Pidsley and Mill 2011). Thus, the mechanisms for gene-environment interaction are largely unknown, but one possible explanation is epigenetics. Epigenetics is defined as the study of mitotically heritable, but potentially reversible, changes in gene expression that occur without a change in the genomic DNA sequence, brought about principally through alterations in DNA methylation and chromatin structure (Henikoff and Matzke 1997). DNA methylation involves the transfer of a methyl group to position 5 of the cytosine pyrimidine ring (C) of a cytosine-guanine dinucleotide (CpG), a reaction catalysed by a group of enzymes called DNA methyltransferases (DNMTs) (Klose and Bird 2006). The methylation of CpG sites acts to disrupt the binding of transcription factors and attract methyl-binding proteins that initiate chromatin compaction and gene silencing (Klose and Bird 2006). Also, methylation and acetylation of histone proteins in the nucleus play an important role in regulating gene expression. These alterations in the structure of histone proteins affect the access of the cell′s transcriptional machinery to the DNA, and consequently the transcription of DNA.

It has been suggested that epigenetic factors play a role in schizophrenia. The evidence for epigenetic abnormalities in schizophrenia in part comes from the study of schizophrenia candidate gene regulation, including GAD67 and reelin (Gavin and Sharma 2010). A decrease in the GAD67 enzyme leading to reduced production of GABA from glutamate in GABAergic interneurons is thought to cause perturbation of cortical activity and the working memory deficits observed in schizophrenia (Lewis et al. 2005). Reelin most likely has a role in synaptic plasticity and learning and memory formation. A deficit in reelin likely accounts for the decreased numbers of dendritic spines in postmortem brains of schizophrenia subjects (Guidotti et al. 2005). There are several reports showing that these genes are downregulated in schizophrenia (Guidotti et al. 2000, Fatemi et al. 2005). The promoters of both GAD67 and reelin are located within CpG islands and, therefore, are especially prone to regulation through DNA methylation (Gavin and Sharma 2010). Chen et al. (2002) have shown in neural precursor cells that increased promoter CpG

island methylation associates with decreased expression of the reelin gene. Several postmortem studies have supported the increased DNA methylation hypothesis. There has been reported increased levels of S-adenosylmethionine (SAM), which serves as a methyl donor in the DNA methylation process (Guidotti et al. 2007) and increased DNA methylation at the reelin promoter (Abdolmaleky et al. 2005) in schizophrenia patient samples. On the other hand, it has been reported that cortical interneurons overexpress DNMT in psychosis, leading to increased methylation of DNA (Veldic et al. 2005). It is likely that this overexpression of DNMT and downregulation of GAD67 and reelin are functionally linked in psychosis. In addition, aberrant histone modifications have been shown in schizophrenia. Two studies have detected elevated levels of histone deacetylase 1 (HDAC1) in schizophrenia postmortem brain samples (Benes et al. 2008, Sharma et al. 2008). Sharma et al. (2008) have shown a correlation between higher levels of HDAC1 expression and reduced GAD67 expression.

Epigenetic mechanisms, such as chromatin remodeling and DNA methylation, are amenable to pharmacological intervention; therefore, there is a potential to develop medications which modify these epigenetic processes. Theoretically, DNMT1 hyperactivity in psychotic patients could be corrected with drugs that directly or indirectly reduce DNMT1 activity or reduce the elevated content of SAM. This could lead to reduction of the DNA hypermethylation observed in putative candidate gene promoters in cortical GABAergic neurons in psychotic patients (Guidotti et al. 2007). An existing example of a drug that affects epigenetic processes is mood stabiliser valproic acid, which is a weak inhibitor of histone deacetylase (Costa et al. 2002). Epigenetic studies have several limitations, one of which is the need for post-mortem human brain tissue, because epigenetic changes can be tissue- and cell-specific and so are most likely to be apparent at the site at which the disorder is primarily manifest (Pidsley and Mill 2011). Despite extensive research efforts the roles of genetic and environmental factors in the genesis of psychoses are poorly understood. Epigenetics has the potential to integrate and mediate the effect of genetic and environmental factors and it has potential therapeutic implications; therefore, it is probable that its importance in psychosis research will grow in the future.

## 2.10 Genetic special features of the Finnish population

Finland has been inhabited for about 10,000 years since the last glacial period. The coastal regions were firstly inhabited. Thereafter, two major migration waves have had a major effect on the gene pool of current Finns. The first wave came about 4000 years ago from South Russia and the second came from Central Europe approximately 2000 years ago. For centuries, only the coastal regions of Finland were inhabited and no major migration to northern and eastern Finland occurred before the 1500s (Early Settlement). The sixteenth century saw a third major migratory movement, especially from South Savo to inland (Late Settlement). That movement forced the Saami population to move further to the north, and no major genetic admixture is thought to have occurred between these subpopulations. The result of the late settlement was that nearly the whole of Finland was inhabited by the end of the 1600s. However, it is remarkable that the inhabitant groups remained small and isolated even until World War II. This has had an effect on both the genetic substructures and regional prevalences of many disorders. An example of this is an internal isolate in the northeastern part of Finland, where the lifetime risk of schizophrenia is 3.2% compared to 1.1% in the rest of Finland (Hovatta 1997).

Two factors that have affected the Finnish gene pool are the genetic drift and genetic bottlenecks. The genetic drift means the random sampling of gene variants into different settler groups and changes in their frequencies over time. A genetic bottleneck is an evolutionary event in which a significant percentage of a population is killed or otherwise prevented from reproducing. Genetic bottlenecks, such as famines, wars and infectious diseases, reduce the genetic variation. An example of a genetic bottleneck in Finland is the great famine during 1696–1698 that killed about a third of the population. The long-lasting isolation of the Finnish population with only minor external migration has led to the expansion of the population having resulted mostly from population growth. This founder effect, arising from the small number of the original settlers, also reduces genetic variation in the Finnish population. Therefore, the Finns and other isolated populations are often called founder populations. The limited allelic variation and relatively uniform genetic background of the Finns are considered beneficial in genetic studies, because they help to avoid a population stratification error. Population stratification means that in the study sample there are subpopulations with different allelic frequencies that can lead to biased or spurious results, for example, false positive discoveries (Cardon and Palmer 2003).

# 3. AIMS OF THE STUDY

This study was designed to elucidate the pathogenetic mechanisms of schizophrenia on the basis of the neurodevelopmental and dopamine theory by investigating the associations between gene polymorphisms of certain growth factors, inflammatory cytokines, dopamine receptor and schizophrenia using the candidate gene approach. The specific aims were:

1. To study the association between the IL-1 gene complex polymorphism and the risk of schizophrenia (I).

2. To investigate the association between the TNF-α –G308A polymorphism and the risk of schizophrenia and the age of onset of the disease (II).

3. To study the association between the EGF A61G polymorphism and the risk of schizophrenia and the age of onset of the disease (III).

4. To test the association between the DRD2 C957T polymorphism and the risk of schizophrenia and the age of onset of schizophrenia (IV).

5. To investigate IL-1β gene polymorphism and its interactions with the neuregulin-1 gene polymorphism in schizophrenia (V).

# 4. SUBJECTS AND METHODS

## 4.1 Patient population

The patients for this study were recruited between 1st January 1997 and 31st December 2000. The patient population consisted of schizophrenia patients meeting the DSM-IV criteria. The patient group consisted of two separate sub-groups. In the first sub-group (Helsinki/Lappeenranta group) there were 50–149 inpatients (I-V) from the psychiatric departments of Helsinki University Hospital and South Karelia Central Hospital. The following exclusion criteria were applied in the Helsinki/Lappeenranta group: another axis I diagnosis (substance abuse, organic mental disorder, affective disorder) and neurological illness. In this group an experienced psychiatrist interviewed all of the patients, evaluated the hospital records, and checked the diagnosis based on a Structured Clinical Interview for DSM-IV Axis I Disorders – clinician version (SCID-I/CV) (First et al. 1997). In the second sub-group (Tampere group) there were 94 patients (IV) who were or had been in the psychiatric departments of Tampere University Hospital. Exclusion criteria in the Tampere group were as follows: major affective disorder, schizo-affective disorder, drug or alcohol abuse, neurological or somatic disease likely to cause psychotic symptoms. In this group an experienced psychiatrist interviewed all of the patients and checked the diagnoses according to the DSM-IV criteria by evaluating the hospital records.

The age of onset was defined in the Helsinki/Lappeenranta group as the first occurrence of positive psychotic symptoms (Meltzer et al. 1997). This information was acquired from the patient´s medical records and from interviews with both the patients and their relatives. In the Tampere group the age of onset was determined

as the patient´s age during the first hospitalisation at which the diagnosis of schizophrenia or schizophreniform psychosis was used. This data was obtained from the Hospital Discharge Register.

Unfortunately, we do not have exact data of those who fulfilled the inclusion criteria but declined to participate in the study. However, it can be estimated that their proportion was approximately 20–25% of the patients. The most common reason for refusing was suspiciousness.

**Study I.** The patient group consisted of 50 schizophrenic inpatients (34 males, 16 females, age range 19–61 years) from the psychiatric departments of Helsinki University Hospital and South Karelia Central Hospital. Twenty patients met the criteria for paranoid, 19 for disorganised, and 11 for the undifferentiated form of acute or chronic schizophrenia.

**Study II.** In this study there were 149 inpatients (age range 18–76 years; mean age (±SD) 38.7±12.2 years) from the psychiatric departments of Helsinki University Hospital and South Karelia Central Hospital. The patient population was an extension of the patient group in Study I. There were 55 women and 94 men. Sixty-one patients met the criteria for paranoid, 47 for undifferentiated, 35 for disorganised, three for residual, and three for a catatonic form of acute or chronic schizophrenia. The mean age (±SD) of onset of schizophrenic psychosis was 23.6±6.3 years. The mean duration (±SD) of schizophrenia was 15.4±11.4 years. At the time of enrollment, 57 patients were being treated with clozapine, 52 patients with typical antipsychotic medication, and 31 patients with atypical antipsychotic medication other than clozapine. In nine cases the information of medication was lacking. The family background of the patients was as follows: 34 patients had first-degree relatives with confirmed schizophrenia, 17 patients had schizophrenia in the extended family history (no first-degree relatives), 77 patients had no schizophrenic relatives, and in 21 patients the family background was undefined.

**Study III.** The patient group was the same as in Study II.

**Study IV.** The patient population consisted of 188 patients (age range 18–76 years; mean age (±SD) 43.1±12.0 years). There were 85 women and 103 men. Con-

cerning the definition of the age of onset we had two subpopulations of patients. In the first group (Helsinki/Lappeenranta group) there were 94 inpatients from the psychiatric departments of Helsinki University Hospital and South Karelia Central Hospital and their mean age of onset was 24.4±6.4 years, as defined as the first occurrence of psychotic symptoms. This patient group was the same as in Study II (149 patients), of which we included all of the patients that we could successfully genotype. In the second group of 94 patients (Tampere group) the mean age of onset (±SD) was 29.6±10.1 years, as defined as the patient´s age when the diagnosis was first used in hospital. The mean duration (±SD) of schizophrenia in the whole population was 16.1±9.6 years.

**Study V.** The patient sample consisted of 113 patients (age range 18–76 years; mean age (±SD) 39.7±12.0 years) from the psychiatric departments of Helsinki University Hospital and South Karelia Central Hospital. This patient group was the same as in Study II (149 patients), of which we included all of the patients that we could successfully genotype. There were 44 women and 69 men. Forty-eight patients met the criteria for paranoid, 37 for undifferentiated, 22 for disorganised, three for residual, and three for catatonic acute or chronic schizophrenia. The mean age (±SD) of onset of schizophrenic psychosis was 23.7±6.1 years. The mean duration (±SD) of schizophrenia was 16.3±11.2 years. All patients had been hospitalised because of acute or chronic schizophrenic psychosis.

The gender distribution and the mean age of onset of schizophrenia of the patients in Studies I–V are shown in Table 8.

**Table 8.** Gender distribution of the patients and mean age of onset.

| | Study 1 | Study 2 | Study 3 | Study 4 | Study 5 |
|---|---|---|---|---|---|
| Male N | 34 (68.0%) | 94 (63.1%) | 94 (63.1%) | 103 (54.8%) | 69 (61.1%) |
| Female N | 16 (32,0%) | 55 (36.9%) | 55 (36.9%) | 85 (45.2%) | 44 (39.9%) |
| Total N | 50 (100%) | 149 (100%) | 149 (100%) | 188 (100%) | 113 (100%) |
| Age of onset ±SD (years) | 22.5±6.1 | 23.6±6.3 | 23.6±6.3 | 24.4±6.4[1]<br>29.6±10.1[2] | 23.7±6.1 |

[1] Helsinki/Lappeenranta group
[2] Tampere group

The overlap of patients between Studies I–V is presented in Table 9.

**Table 9.** The overlap of patients between Studies I–V.

| | Study I (IL-1, n=50) | Study 2 (TNF-α, n=149) | Study 3 (EGF, n=149) | Study 4 (DRD2, n=188) | Study 5 (IL-1β+NRG, n=113) |
|---|---|---|---|---|---|
| Study I (IL-1, n=50) | - | 50 | 50 | 5 | 16 |
| Study 2 (TNF-α, n=149) | 50 | - | 149 | 94 | 113 |
| Study 3 (EGF, n=149) | 50 | 149 | - | 94 | 113 |
| Study 4 (DRD2, n=188) | 5 | 94 | 94 | - | 94 |
| Study 5 (IL-1β+NRG, n=113) | 16 | 113 | 113 | 94 | - |

## 4.2 Controls

The control groups consisted of 94 (49 males, 45 females) (III), 384 (206 males, 178 females) (IV), 393 (213 males, 180 females) (II, V) and 400 (218 males, 182 females) (I) healthy blood donors from the Finnish Red Cross. The overlap of healthy controls between Studies I–V is shown in Table 10.

**Table 10.** The overlap of healthy controls between Studies I–V.

| | Study 1 (IL-1, n=400) | Study 2 (TNF-α, n=393) | Study 3 (EGF, n=94) | Study 4 (DRD2, n=384) | Study 5 (IL-1β+NRG, n=393) |
|---|---|---|---|---|---|
| Study 1 (IL-1, n=400) | - | 393 | 94 | 384 | 393 |
| Study 2 (TNF-α, n=393) | 393 | - | 94 | 384 | 393 |
| Study 3 (EGF, n=94) | 94 | 94 | - | 93 | 94 |
| Study 4 (DRD2, n=384) | 384 | 384 | 93 | - | 384 |
| Study 5 (IL-1β+NRG, n=393) | 393 | 393 | 94 | 384 | - |

Before each donation the subjects complete a written health statement, including information on medication, allergies, infectious diseases, heart diseases, other chronic

diseases, and mental health. In addition, a qualified nurse interviews the subjects before each donation session on the basis of the health questionnaire and measures the blood haemoglobin of the donor. Blood donors are not paid in Finland.

## 4.3 Genotyping methods

From all patients, 10 ml of venous blood was drawn into EDTA vacuum tubes and immediately frozen at -20 degrees Celsius for later DNA isolation. Genomic DNA was extracted from peripheral blood leukocytes of the controls using a commercially available kit (Qiagen Inc., Hilden, Germany) and from venous blood of the patients using the salting-out method (Miller et al. 1988). Genotypings were performed in the Department of Clinical Chemistry and in the Department of Microbiology and Immunology, University of Tampere Medical School and Tampere University Hospital.

### 4.3.1 Interleukin-1 gene complex genotyping (I, V)

The genotyping of the IL-1RA (VNTR, rs380092) polymorphism was performed as described earlier (Tarlow et al. 1993). Briefly, oligonucleotides were used as primers in a polymerase chain reaction (PCR). Conditions used were: 96°C for 1 min, followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, 70°C for 1 min, and finally 72°C for 5 min. The PCR products were analysed by electrophoresis on a 2% agarose gel stained with ethidium bromide.

The genotyping of the IL-1β (-511) polymorphism (rs16944) was carried out as follows: the region that contains the AvaI polymorphic site at position -511 of the IL-1β gene (diGiovine et al. 1992) was amplified by PCR. The oligonucleotides flanking this region were used as primers. PCR conditions were as follows: 95°C for 2 min, 55°C for 1 min, 74°C for 1 min, and then 38 cycles of 95°C for 1 min, 55°C for 1 min, 74°C for 1 min, and finally 74°C for 4 min. The products were digested with six units of AvaI at 37°C for 3 h. Fragments were analysed by electrophoresis on 9% polyacrylamide gel electrophoresis (PAGE), stained with ethidium bromide. This gave products of 190 bp + 114 bp (allele 1) and 304 bp (allele 2).

For the IL-1α (-889) genotyping (rs1800587) we amplified the polymorphic region of the IL-1α as previously described (McDowell et al. 1995) using oligo-

nucleotide primers based on the DNA sequences that flank the polymorphic site. Conditions used were a denaturing step of 96°C for 1 min and then 40 cycles of 94°C for 1 min, 51°C for 1 min, 72°C for 1 min, and finally 72°C for 4 min and 55°C for 5 min. The products were digested with NcoI and the resultant products were analysed on 9% PAGE. This gave products of 83 bp + 16 bp (allele 1) and 99 bp (allele 2).

### 4.3.2 Tumour necrosis factor-alpha, epidermal growth factor, dopamine-2 receptor and neuregulin-1 genotyping (II-V)

Tumour necrosis factor-alpha –G308A (rs1800629), epidermal growth factor A61G (rs4444903), dopamine-2 receptor C957T (rs6277) and neuregulin-1 SNP221533 (rs35753505) genotypes were determined using fluorogenic allele-specific oligonucleotide probes with the conjugated minor groove binder (MGB) group (Livak 1999). The nucleotide sequences of the primers and probes used in the PCR were deduced from the published sequences deposited in the GenBank database and chosen and synthesised in conjunction with Applied Biosystems (Foster City, CA, USA), using the Assay-by-Design tool. The reporter dyes chosen were VIC (G-specific probe) and FAM (A-specific probe). DNA samples were genotyped by employing the 5' nuclease assay for allelic discrimination using either the ABI Prism 7000 or 7900HT Sequence Detection Systems (Applied Biosystems, Foster City, CA, USA). A PCR reaction containing genomic DNA, 1 x Universal PCR Master Mix, 900 nM of each primer, and 200 nM of each probe was performed in 96-well plates using the standard protocol for TaqMan MGB probes in a total volume of 25 µl. Water controls and known control samples previously (for TNF-α SNP rs1800629 and EGF SNP rs4444903) typed by RFLP-PCR analysis were run in parallel with unknown DNA samples. After cycling, end-point fluorescence was measured and genotype calling was carried out using the allelic discrimination analysis module.

## 4.4 Data analysis

The Pearson chi-square test was used to compare the frequency distributions of the genotypes and alleles between the schizophrenic patients and healthy controls. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated to evaluate the

effects of different genotypes/alleles. Bonferroni correction was applied in Study I to correct for multiple testing.

A student´s t-test and one-way ANOVA were used to compare the age of onset of schizophrenia between different genotypes. The Kaplan-Meier method and the log rank test for the analysis of survival were used to study the association between the age of onset and the gene polymorphism (Studies III and V).

The interaction analyses in Study V were performed with a logistic regression model and odds ratios and 95% confidence intervals were calculated with the –2log likelihood test.

The power analysis in Study II was made with a power and sample size calculation program (PS program version 2.1.30).

The statistical calculations were performed by using the Statistica software (StatSoft Inc., Tulsa, OK, USA) (I) and the SPSS version 12.0 (SPSS Inc., Chicago, Illinois, USA) (II-V) on a microcomputer. In all studies the level of statistical significance was defined as $P<0.05$.

## 4.5 Ethics

The investigators assessed the patients being capable of understanding the study procedure after its nature had been fully explained in the interview and in a written description of the study given to them. Thereafter, all patients gave written informed consent for the study. The ethics committees of all three hospitals had approved the study. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

# 5. RESULTS

## 5.1 Interleukin-1 gene complex polymorphism in schizophrenia (I)

We found in our material of 50 patients with schizophrenia and 400 healthy controls that the number of carriers of the allele combination of IL-1A (-889) (rs1800587) allele 2 positive, IL-1B (-511) (rs16944) allele 2 negative, and IL-1RN (VNTR, rs380092) allele 2 negative was significantly higher in the patient group (P=0.026). In addition, the frequency of the allele combination of IL-1A (-889) allele 2, IL-1B (-511) allele 1, and IL-1RN (VNTR) allele 1 was higher in schizophrenic patients (P=0.0266). The number of IL-1A allele 2, IL-1B allele 1, and IL-1RN allele 1 homozygotes was clearly higher in schizophrenic patients than in controls (N=8/50 in cases vs 17/400 in controls) (P=0.0006).

The frequencies of the IL-1A allele 2, IL-1B allele 1 and IL-1RN allele 1 tended to be somewhat, but not significantly, higher in the schizophrenia group (P=0.72, P=0.06 and P=0.66, respectively).

## 5.2 Tumour necrosis factor alpha polymorphism in schizophrenia (II)

We found in our sample of 149 patients with schizophrenia and 393 healthy controls that the allele and genotype frequencies did not differ significantly between the patient and control groups (P=0.10 and 0.12, respectively), but the frequency of TNF-α –G308A G/G (rs1800629) homozygotes was higher in male patients than in male controls (pointwise P=0.025; odds ratio (OR) 2.00, 95% CI 1.08–3.70). In female patients or in the whole study group, no such difference was seen.

The age of onset of schizophrenia did not differ between the different TNF-α genotypes (P=0.64).

## 5.3 Epidermal growth factor polymorphism in schizophrenia (III)

Our study with 149 schizophrenic patients and 94 healthy controls showed that the EGF A61G polymorphism (rs4444903) was significantly associated with the age of onset of schizophrenia in male patients. The mean ages (±SD) of onset in male G/G homozygotes and other genotypes were 20.1±3.9 and 23.7±6.6 years, respectively (Student´s t-test P=0.022; Kaplan-Meier log rank test P=0.005). A difference in the age of onset of schizophrenia between G/G homozygotes and other genotypes was also seen in the entire patient population, but not in female subjects (Kaplan-Meier log rank test P=0.008 and P=0.46, respectively).

No significant differences in the allele and genotype frequencies were detected between the patients and the controls (difference in the allele and genotype frequencies, P=0.61 and P=0.89, respectively).

## 5.4 Dopamine-2 receptor polymorphism in schizophrenia (IV)

We found in our material of 188 patients with schizophrenia and 384 healthy controls that the DRD2 C957T polymorphism (rs6277) had an effect on the risk of schizophrenia: in all subjects the C-allele carrier status (C/T+C/C) increased the risk of belonging to the patient group (P=0.050; OR 1.5, 95% CI 1.0–2.3). In addition, homozygote enrichment analysis on the C/C and T/T genotypes with maximal functional difference in DRD2 binding (Hirvonen et al. 2004) indicated that the

C/C genotype, as compared to the T/T genotype, was significantly associated with schizophrenia (P=0.0086; for genotype distribution C/C, C/T and T/T between patients and controls, P=0.14).

There was no significant difference in the allele distributions between the schizophrenic patients and the controls, although the C-allele tended to be more common in the patient group (P=0.06).

The mean age of onset did not differ between the different DRD2 genotypes (ANOVA, F=0.91, P=0.41).

## 5.5 Interleukin-1β polymorphism and its interactions with neuregulin-1 polymorphism in schizophrenia (V)

We found in a sample of 113 schizophrenic patients and 393 healthy controls a significant interaction between the IL-1B (-511) (rs16944) and neuregulin-1 SNP221533 (rs35753505) polymorphisms concerning the risk of schizophrenia. The risk of having schizophrenia was more than 10 times higher (OR 10.20, 95% CI 2.53–41.09, pointwise P=0.001) when having the IL-1B 2/2, NRG-1 C/C genotype (N=7/113 patients vs. 5/393 controls) instead of the IL-1B 2/2, NRG-1 T-allele carriage (N=7/113 patients vs. 51/393 controls). In addition, the interaction between IL-1B and NRG-1 polymorphisms was trend-like associated with the age of onset of schizophrenia (P=0.09, log rank test). In the patients with the combination of the IL-1B allele 1 carriage and NRG-1 C/C homozygotism (N=12/113 patients) the age of onset of the disease was later than with other allele combinations (P=0.027, t-test).

IL-1B -511 allele 1 homozygotes showed a higher age of onset of schizophrenia than the allele 2 carriers (P=0.012, log rank test).

The NRG-1 polymorphism did not associate with the age of onset of schizophrenia (ANOVA, F=0.79, P=0.46).

There were no differences in IL-1B or NRG-1 genotype distributions between schizophrenic patients and controls (P=0.53 and P=0.66, respectively). Nor were there any differences in IL-1B or NRG-1 allele frequencies between patients and controls (P=0.28 and P=0.36, respectively).

**Table 11.** Summary of the results.

| Gene polymorphism | IL-1A | IL-1B | IL1-RN | Combination IL-1A, IL-1B and IL-1RN | TNF-alpha | EGF | DRD2 | Combination IL-1B and NRG-1 |
|---|---|---|---|---|---|---|---|---|
| | -889 | -511 | VNTR | | G308A | A61G | C957T | -511 and SNP221533 |
| association with risk of schizophrenia | - | - | - | + | + | - | + | + |
| male | - | - | - | + | + | - | - | + |
| female | - | - | - | - | - | - | - | - |
| association with age of onset | not studied | not studied | not studied | not studied | - | + | - | +[1] |
| male | | | | | - | + | - | - |
| female | | | | | - | - | - | - |

[1] only IL-1B polymorphism

# 6. DISCUSSION

## 6.1 Methodological considerations

### 6.1.1 Selection of study subjects

The purpose of this study was to investigate the pathogenetic mechanisms of schizophrenia on the basis of the neurodevelopmental and dopamine theory by studying the associations between gene polymorphisms of certain growth factors, inflammatory cytokines, dopamine receptor and schizophrenia. All of the patients were admitted to a psychiatric hospital for acute or chronic schizophrenic psychosis. The patients were severely ill and a substantial portion of them were considered treatment-resistant and, therefore, used clozapine medication. The mean duration ± SD of schizophrenia was about 16 ±11 years, which indicated that the study population was rather chronic in nature. To gain as homogenous a patient sample as possible, we applied strict inclusion and exclusion criteria. The inclusion criteria were that the patient had to have a schizophrenic psychosis meeting the DSM-IV criteria for schizophrenia and that the patient had to be over 18 years old. The diagnoses were assigned based on a Structured Clinical Interview for DSM-IV Axis I Disorders – clinician version by experienced psychiatrists (First et al. 1997). We excluded the patients whose psychosis may have resulted from an additional Axis I diagnosis (substance abuse, organic mental disorder, affective disorder), or neurological illness.

At the time of the patient collection in the late 1990s there were only a small amount of patients with a concomitant substance problem (double-diagnosis) and which were therefore to be excluded. Thus, the selection bias because of excluding the double-diagnosis patients is probably not large, but it could have led to some selection bias, especially in the male study population. Another possible cause of selection bias is that some psychotic patients expressed fear of genetic studies and did not give their permission for the study. The same was also seen with some paranoid patients. On the whole, people disposed positively towards the study and about 80% of the interviewed patients agreed to participate in the study.

### 6.1.2 Selection of genetic polymorphisms

The neurodevelopmental and the dopaminergic hypotheses are among the leading theories concerning the etiopathogenesis of schizophrenia. We were primarily interested in studying genes of which there was evidence for a role either in neurodevelopment or in dopaminergic transmission and possibly research data showing aberrant expression in schizophrenia patients.

Former studies have provided evidence of abnormal levels of cytokines of the IL-1 cluster in the peripheral blood of schizophrenic patients, indicating dysregulation of these cytokines in schizophrenia (Katila et al. 1994). There are also reports showing that genetic polymorphisms of the IL-1 cluster have an effect on the levels of these cytokines (Hurme and Santtila 1998). Because of the biological evidence of the possible role of the IL-1 cluster genetic polymorphisms in schizophrenia we decided to select this gene group for our study. At the time of our study there were no reports concerning the IL-1 gene cluster polymorphisms and schizophrenia.

Elevated levels of proinflammatory cytokine TNF-α have been detected in peripheral blood of schizophrenic patients (Ganguli et al. 1994). There is also evidence that these cytokines have effects on the survival of neurones (Araujo and Cotman 1995, Marx et al. 2001) and on formation of neurites and neuronal survival, and maintaining synaptic strength in the CNS (Marx et al. 2001, Beattie et al. 2002, Neumann et al. 2002). There have been several recent studies concerning the possible association between the TNF-α -G308A polymorphism and schizophrenia but the results are remarkably inconsistent, including both positive (Boin et al. 2001, Meira-Lima et al. 2003, Schwab et al. 2003b) and negative findings (Riedel et al. 2002, Handoko et al. 2003). A recent meta-analysis of 21 studies with a total of

4340 patients and 5745 controls did not show any association between the TNF-α –G308A polymorphism and schizophrenia (Qin et al. 2013). It is speculated that the differences between the studies could be due to genetic heterogeneity between the different study populations. We selected the TNF-α gene for our study to investigate the possible role of the G308A polymorphism of this gene for schizophrenia in a Finnish population.

EGF is a polypeptide which is expressed in the brain and studies suggest that it has an effect on neurodevelopment by regulating the activities of neuronal and glial progenitor cells in the developing brain in vivo (Seroogy et al. 1995). Decreased EGF levels have been detected in schizophrenia patients (Futamura et al. 2002). Shahbazi et al. (2002) reported that an SNP of the EGF gene, G61A, had an influence on the production of EGF in vitro. Anttila et al. (2004) studied the EGF G61A genetic polymorphism in 94 Finnish patients with schizophrenia and found that the G allele was associated with schizophrenia in male patients. The G allele was also associated with a later age of onset in male patients (Anttila et al. 2004). Our aim was to replicate Anttila et al.´s (2004) results in a larger independent schizophrenia population; therefore, we chose the EGF G61A genetic polymorphism for our study.

NRG-1 is a pleiotropic growth factor with effects on cell fate determination, interneuron migration, myelination, receptor recruitment, and synaptic plasticity (Harrison 2007). The first evidence that the NRG-1-erB signalling is altered in schizophrenia came from the postmortem studies by Hakak et al. (2001) which showed significantly decreased expression of erbB3 in the dorsolateral prefrontal cortex of chronic schizophrenic patients compared to controls. A genome-wide scan of a large Icelandic sample revealed the NRG-1 gene as a susceptibility locus for schizophrenia (Stefansson et al. 2002). The same group also found in this region an NRG-1 at-risk haplotype of seven markers and one SNP in the 5′ region of the NRG-1 gene that was significantly different in schizophrenic patients compared to controls. NRG-1 is a growth factor which has a broad range of bioactivities with effects on neurodevelopment and glutamatergic and dopaminergic neurotransmission, whose aberrations are suggested to be important in the etiopathogenesis of schizophrenia (Carlsson et al. 1999, Harrison 2007). This makes NRG-1 an interesting candidate gene for schizophrenia susceptibility.

Imbalances of the dopaminergic neurotransmission of the CNS have been suggested to have a role in the etiopathogenesis of schizophrenia since the 1960s (Carlsson and Lindqvist 1963). This dopaminergic hypothesis has been supported

by pharmacologic research (Seeman and Lee 1975, Creese et al. 1976). Despite experimental data supporting this hypothesis, documentation of abnormalities of dopamine function in schizophrenia has remained elusive. Over the last few years, progress in brain imaging methods has enabled direct measurement of dopamine transmission at D2 receptors, and the application of these techniques to the study of schizophrenia has provided new insights into the nature and the role of dopamine function aberrations in this illness. In vitro studies of the DRD2 have shown that one SNP of its gene, C957T, has marked functional consequences for DRD2 mRNA stability and dopamine-regulated DRD2 expression, probably by affecting the secondary structure of mRNA (Duan et al. 2003). In a recent Finnish study it was reported that C957T affects striatal D2 binding in healthy humans (Hirvonen et al. 2004). This new brain imaging data gave us the idea to study the possible association between the DRD2 C957T polymorphism and schizophrenia.

## 6.2 Genetic polymorphisms and risk of schizophrenia

The main aim of this study was to investigate the etiology and the genetic background of schizophrenia by finding genes whose variation would be associated with the risk of schizophrenia (susceptibility gene) or the age of onset of the illness. In addition, we studied the possible interaction between two neurodevelopmental genes, IL-1B and NRG-1, and its effect on the risk and the age of onset in schizophrenia. In the literature, there is variable evidence of several schizophrenia susceptibility genes. Among the best replicated susceptibility genes are NRG-1, DTNBP1, DISC1, COMT, G72, and RGS4 (Harrison and Weinberger 2005, Williams et al. 2009, Doherty et al. 2012).

NRG-1 is one of the strongest candidates for a susceptibility gene for schizophrenia with multiple positive associations and two positive meta-analyses (Harrison and Law 2006). There are also several other factors supporting the suggestion that NRG-1 is related to the etiology of schizophrenia. Firstly, there is prior evidence for linkage to chromosome 8p, where the NRG-1 locus lies (Stefansson et al. 2002). Secondly, in NRG-1 mutant mice there is a schizophrenia-like phenotype (Stefansson et al. 2002). Thirdly, the known functions of NRG-1 (for example: neuronal specification, migration, synapse formation, dendritic growth, transcriptional regulation) are relevant to the disease process in schizophrenia (Corfas et al. 2004). Fourthly, there are findings that NRG-1 expression is altered in schizophrenia and related to

the risk of schizophrenia (Harrison and Law 2006). However, there are two caveats that must be taken into account when assessing NRG-1 as a susceptibility gene for schizophrenia. Firstly, amongst the positive association studies, many different alleles and haplotypes have been associated with schizophrenia. Possible explanations for this could be false positive findings or publication bias. Some of the differences could be explained by ethnical differences: in most studies with Caucasians, an association has been found with the region around the first two exons of the gene, whereas some Chinese populations show an association with more 3′ markers (Harrison and Law 2006). Secondly, all of the NRG-1 polymorphisms associated with schizophrenia are non-coding, apart from two with an Arg-Gly substitution. Harrison and Law (2006) suggest that a likely explanation for this could be that the current polymorphism is in linkage disequilibrium with the "true" NRG-1 gene variant.

There is evidence of an association between the SNP NRG-1 SNP8NRG221533 polymorphism and schizophrenia (Stefansson et al. 2002, Yang et al. 2003). In our study, we found no association between the NRG-1 SNP8NRG221533 genotype or allele frequencies and schizophrenia. This agrees with the results of an earlier study with Finnish schizophrenic patients (Kampman et al. 2004). It is thus possible that this polymorphism is not associated with schizophrenia in Finnish patients, although the sample size was small and quite limited in power to detect small/modest differences in allele or genotype frequencies. Another possibility is that there is a type II error (false negative) in both studies, because of the relatively small sample size in both studies. However, it is remarkable that our results are parallel with the recent meta-analysis by Munafo et al. (2006), who discovered no evidence of an association with the 5′ region SNP (SNP8NRG221533). Moreover, in the most recent analysis of the NRG-1 polymorphisms in schizophrenia in Finland, Turunen et al. (2007) did not find any association between the SNPs of the 5' end of NRG and schizophrenia, but their results give weak support for the involvement of the 3′ region of NRG-1 in the etiology of the illness.

Our results concerning the EGF A61G polymorphism showed no significant differences in genotype or allele frequencies between the patient and control groups, suggesting that this polymorphism does not affect the risk of schizophrenia. This is well in line with the previous studies by Lim et al. (2005) and Watanabe et al. (2005), but we could not confirm the previous finding of an association between the G allele and schizophrenia in male patients (Anttila et al. 2004). Futamura et al. (2002) found reduced EGF levels in the peripheral blood of schizophrenic patients. Combined with the finding that A/A homozygotes have low levels of EGF produc-

tion (Shahbazi et al. 2002), it could be suggested that A/A homozygotes would be more common among schizophrenics than controls. This is not in line with our study. One explanation could be that schizophrenics smoke more than people on average, and smoking reduces EGF levels (Ma et al. 2000). Furthermore, several other factors, such as drugs, hormones, systemic diseases, ageing, and emotional stress, affect serum EGF levels (Plata-Salamán 1991, Apostolakis et al. 2000, Koller et al. 2000, Brannvall et al. 2002, Pareja et al. 2003). In addition, Hashimoto et al. (2005b) found no changes in serum EGF levels in patients with schizophrenia.

Our study of the TNF-α -G308A polymorphism did not reveal significant differences in the genotype and allele frequencies between the patients and controls in the whole patient group. This result parallels with several previous or more recent studies (Riedel et al. 2002, Tsai et al. 2003, Kampman et al. 2004, Pae et al. 2006, Shirts et al. 2006a, Zai et al. 2006, Watanabe et al. 2007a). Boin et al. (2001) reported the frequency of the -308A allele to be significantly increased in Caucasian Italian schizophrenic patients compared with controls with the same ethnicity. Genotype distribution was also significantly different between schizophrenics and controls in their study. It is remarkable that in Boin et al.'s (2001) study the frequency of the A allele in the control group (0.11) was much lower than in the other published Caucasian controls (0.16–0.19). In addition, the inclusion criteria were different between our study and Boin et al.'s (2001) study because the latter also included patients with schizoaffective disorder (14.3% of all patients), while we had only patients with a core diagnosis of schizophrenia. Moreover, most of our patients were severely ill and many of them were considered treatment-resistant with 38% on clozapine medication. It is possible that our different results, compared with earlier studies, indicate that the most severely ill patients, with their poor response to neuroleptic medication, may constitute a pharmacogenetically distinct subgroup of schizophrenia. Our study with no association between the TNF-α –G308A polymorphism and schizophrenia is also well in line with another report with an even higher proportion of clozapine-medicated patients (Tsai et al. 2003).

Although we found no association between the TNF-α –G308A polymorphism and schizophrenia in general, there was a gender difference: G/G homozygotes were more common in our male schizophrenic patients than in the healthy male controls, whereas females displayed no differences between the patients and controls in this respect. The reason for this is unclear, but one possible explanation for the gender difference could be the different estrogen levels in males and females. Reduced synaptic connectivity has been suggested to have an important role in the

etiopathogenesis of schizophrenia (McGlashan and Hoffman 2000). TNF-α has been shown to enhance synaptic efficacy and to be required for the preservation of synaptic strength at excitatory synapses (Beattie et al. 2002). In vitro studies have shown that TNF-α A-allele carriers produce more TNF-α than G/G homozygotes (Louis et al. 1998). On the other hand, estrogens have also been shown to influence neural growth, induce synaptogenesis, and delay neural pruning (Woolley et al. 1996, Desmond and Levy 1997, Pozzo-Miller et al. 1999). Thus, it is possible to speculate that the lower TNF-α production in G/G homozygotes, with their possibly weakened synaptic connectivity at excitatory synapses, might be compensated in females with their higher estradiol levels reducing the risk of aberrant functioning at these neural connections.

The DRD2 C957T C-allele was modestly overrepresented among our patients with schizophrenia compared to healthy controls. Additionally, genotypes with the C-allele (C/C and C/T) were more common in patients than in controls. This confirms the results of an earlier study by Lawford et al. (2005), which was performed in Australia with schizophrenia patients of Caucasian origin. It is, however, remarkable that the allele distributions of the control populations in these two studies differed significantly. The reason for this difference is unknown, but may relate to differences in ethnicity of the study participants and to the different genetic origins of Finnish Caucasians compared to Northern European Caucasians living in Australia (Lappalainen et al. 2006). Hoenicka et al. (2006) studied the DRD2 SNP C957T in Spanish schizophrenic patients and also found the C/C genotype and C-allele more frequent in patients than in healthy controls. However, Kukreti et al. (2006) found no association in the observed genotype and allele frequencies for the C957T polymorphism between Indian schizophrenic patients and controls. Monakhov et al. (2008) reported a statistically significant association between the DRD2 C957T polymorphism and schizophrenia, with the C/C genotype being more frequent in Russian schizophrenic patients than in mentally healthy controls. They also performed a meta-analysis based on four previous reports and their work. Their meta-analysis showed a significantly higher frequency of the C/C homozygotism in case samples than in the controls, providing additional evidence of an association of the C957T polymorphism with schizophrenia (Monakhov et al. 2008). Betcheva et al. (2009) performed a case-control study of 183 SNPs in 59 candidate genes with Bulgarian patients with schizophrenia and schizoaffective disorder and healthy controls. They found the DRD2 C957T polymorphism to be the only susceptibility factor for schizophrenia among the tested candidate genes. The C allele

was identified as the risk allele for schizophrenia (Betcheva et al. 2009). Fan et al. (2010) published a case-control association study of DRD2 gene polymorphisms with patients with schizophrenia and healthy controls in a Chinese Han population. They detected an association between the C957T polymorphism (rs6277) and schizophrenia, but contrary to most earlier studies, they found the T-allele to be a risk allele for schizophrenia (Fan et al. 2010). All in all, there is abundant evidence of an association of the C957T polymorphism with schizophrenia and there is also evidence of functional significance of this polymorphism (Duan et al. 2003, Hirvonen et al. 2004) on dopaminergic neurotransmission. Taken together, this offers a theory-based link between gene polymorphism, gene function and the known psychopathophysiology of schizophrenia, and offers an explanation as to why the DRD2 gene can predispose to schizophrenia. Besides the C957T polymorphism, there are also other polymorphisms in the DRD2 gene which have been shown to associate with schizophrenia. Among the most studied DRD2 polymorphisms in schizophrenia are the 141C Ins/Del and Ser311Cys polymorphisms (Sáiz et al. 2010, Liu et al. 2012). Liu et al. (2012) performed a meta-analysis of the DRD2 Ser311Cys polymorphism in an Asian population consisting of 16 studies with a total of 2268 schizophrenia patients and 2423 healthy controls. They demonstrated that the G allele of the DRD2 Ser311Cys polymorphism might be a potential risk factor for schizophrenia in Asian populations, especially in the Japanese population (Liu et al. 2012).

Concerning the IL-1 gene complex, our study showed that the frequency of the genotype combination of IL-1A-889 2/2, IL-1B-511 1/1 and IL-1RN VNTR 1/1 was significantly higher in schizophrenic patients than in controls. Also, the allele combination of IL-1A-889 allele 2, IL-1B-511 allele 1 and IL-1RN VNTR allele 1 was more frequent in patients than in controls. In addition, we found that the number of carriers of the IL-1A-889 allele 2 positive, IL-1B-511 allele 2 negative and IL-1RN VNTR allele 2 negative was higher in patients than in controls. These findings suggest that the cytokine aberrations in schizophrenia are, at least partly, genetically determined. It has been suggested that maternal infection can leave the foetus at a greater risk of developing immunologically mediated brain changes, possibly leading to schizophrenia (Limosin et al. 2003). Therefore, our findings could have importance in understanding etiopathogenesis of schizophrenia. After our study there have been several published reports with a positive association with the IL-1 complex gene polymorphisms and schizophrenia (Zanardini et al. 2003, Kim et al. 2004, Papiol et al. 2004, Sasayama et al. 2011). The results are,

however, conflicting because negative association studies also exist (Chowdari et al. 2001, Saiz et al. 2006, Shirts et al. 2006b, Watanabe et al. 2007b). Mata et al. (2006) investigated the IL-1RN VNTR polymorphism in schizophrenia and found no difference in allele or genotype frequencies between patients and controls, but this polymorphism associated significantly with negative symptom improvement during antipsychotic treatment. Shi et al. (2008) performed meta-analyses of 40 polymorphisms of 12 candidate genes of schizophrenia and found an association between the IL-1B (-511) genetic polymorphism and schizophrenia with Bonferroni correction for the number of meta-analysed polymorphisms in that gene. However, the result was not significant when correction for multiple testing for all of the meta-analyses was conducted.

Because IL-1B and NRG-1 are both candidate genes of schizophrenia and play an important role in CNS development, we studied interactions between the IL-1B-511 and the NRG-1 SNP 221533 in the risk of schizophrenia. We found that the genotype combinations differed significantly with respect to the risk of schizophrenia. The risk combination for schizophrenia was IL-1B 2/2 and NRG-1 C/C genotype with over 10 times higher risk of schizophrenia than the combination of IL-1B 2/2 and NRG-1 T-allele carriage. There is evidence that IL-1B allele 2 is associated with enhanced IL-1α transcription and production (Hurme and Santtila 1998, El-Omar et al. 2000, Chen et al. 2006). On the other hand, MRI studies have demonstrated bifrontal-temporal grey matter volume deficits and generalised white matter deficits in schizophrenic IL-1B-511 allele 2 carriers (Meisenzahl et al. 2001). We speculate that the elevated levels of IL-1α in allele 2 carriers interact with NRG-1 in the developing brain, causing morphological and functional aberrations, which might sensitise an individual to a schizophrenic psychosis. This interaction has not been described earlier in the literature.

## 6.3 Age of onset

The age of onset of schizophrenia varies considerably. The symptoms of schizophrenia usually do not begin until the second and third decades of life, but they sometimes emerge earlier or even as late as the seventh decade (Meltzer et al. 1997). However, in most cases, the initial positive symptoms or disorganisation develop between 15 and 30 years, which strongly suggests that the pathological processes occur in the brain during that period of time (Meltzer et al. 1997). These processes are modulated by

environmental factors and form the final expression of the biological vulnerability factors for schizophrenia. Therefore, identification of the factors affecting the age of onset of schizophrenia can yield information about the biological basis of this illness.

Schizophrenia has been regarded as a disease with a quite heterogeneous origin and evolvement (Fanous and Kendler 2005). Therefore, it is useful to categorise patients with schizophrenia into relative homogeneous subsets based on clinical characteristics including age of onset. Although the pathophysiology of schizophrenia is still unclear, there is increasing evidence supporting a distinct difference between early-onset and adult-onset schizophrenia (Tuulio-Henriksson et al. 2004, Kester et al. 2006, White et al. 2006). Former studies have shown that an earlier onset of schizophrenia is associated with poorer response to treatment (Kolakowska et al. 1985), greater impairment at follow-up (Johnstone et al. 1989), and higher risk of rehospitalisation (Loebel et al. 1992), leading to worse prognosis in these patients.

In our study we found that EGF G/G homozygotes had a significantly earlier onset of schizophrenia than the A allele carriers in male schizophrenic patients (20.1±3.9 vs. 23.7±6.6 years). This is not in line with a previous study by Anttila et al. (2004) with an independent Finnish study population. One possible explanation could be that the definition of the age of onset of the psychosis was clearly different in these two studies. We defined the age of onset as the age at the occurrence of positive psychotic symptoms, whereas Anttila et al.'s (2004) definition for the age of onset was the time of the first hospitalisation of the patient. Lee et al. (2006) studied Korean schizophrenic patients and also found an association between the EGF A61G polymorphism and the age of onset of schizophrenia in male patients. They demonstrated that non-G-allele-carrying subjects were overrepresented in the early-onset subgroup more than the adult subgroup in males. Their results do not parallel with our study, which could be due to ethnic heterogeneity in the EGF polymorphism.

Several studies have implicated glutamatergic dysfunction in schizophrenia (Meador-Woodruff and Healy 2000, Goff and Coyle 2001). A proposed dysfunction of glutamatergic neuronal systems is not inconsistent with the dopamine hypothesis of schizophrenia, because reciprocal synaptic relationships between forebrain dopaminergic projections and glutamatergic systems have been described (Carlsson and Carlsson 1990). EGF has been demonstrated to reduce the release of glutamate from forebrain synaptosomes (Barrie et al. 1996). Shahbazi et al. (2002) have demonstrated that G allele carriers in vitro show significantly more abundant production of EGF than non-carriers. It is thus possible that the abundant EGF production

in G/G homozygote schizophrenics induces an imbalance in dopaminergic and glutamatergic neurotransmission in the brain during the fetal development of the CNS or later in life. This could make a person more vulnerable to environmental stressors, contributing to the early onset of schizophrenic psychosis.

We also found an interaction between the IL-1B -511 and NRG-1 polymorphisms and the age of onset of schizophrenia. We demonstrated that in patients with the combination of the IL-1B allele 1 carriage and NRG-1 C/C homozygotism the age of onset of the disease was later than with other allele combinations, the mechanism of which is not clear. However, it has been demonstrated that IL-1B allele 2 is associated with enhanced IL-1β production (Hurme and Santtila 1998, El-Omar et al. 2000), and in vitro studies have shown that high IL-1β can decrease neuronal survival and may modulate the neuroanatomical alterations that are observed in schizophrenia (Marx et al. 2001). Additionally, MRI studies have revealed bifronto-temporal grey matter volume deficits and generalised white matter deficits in schizophrenic IL-1B-511 allele 2 carriers (Meisenzahl et al. 2001). Taken together, it can be speculated that the elevated levels of IL-1β in allele 2 carriers interact with NRG-1 in the developing brain, causing morphological and functional aberrations which possibly sensitise a person to a schizophrenic psychosis. Correspondingly, IL-1B allele 1 could have a protective effect against schizophrenia by delaying the onset of disease. However, it is to be noticed that the background of our findings has not been clearly verified, and is partly hypothetical. Thus, the results should be viewed cautiously.

According to our results, the TNF-α –G308A polymorphism was not associated with the age of onset of schizophrenia. Our results are parallel with the former study by Riedel et al. (2002). However, in both studies, G/G homozygotes had a lower age of onset than patients with the G/A or A/A genotype, but the difference was not significant.

## 6.4 Limitations and strengths of the study

The small sample size was a limitation of our study, so we could not completely exclude the possibility of type 1 or 2 statistical errors. In post-hoc power calculations the study was somewhat underpowered; thus, the results should be replicated in more powered population samples. Only future studies with independent and preferably larger cohorts will therefore show whether our results can be replicated.

Our patients were mainly severely ill inpatients, many of whom were considered treatment-resistant with clozapine medication. This may make it more difficult to generalise our results to all schizophrenic patients.

There was some selection bias because some schizophrenia patients refused to participate in the study. For the most part, these patients had paranoid fears of the study. However, most patients´ attitudes towards the study were positive. Some selection bias could also be due to our exclusion criteria because we excluded the schizophrenia patients with concomitant substance abuse. These kinds of double-diagnosis patients are more common among males.

A strength of our study is that the patients were selected carefully using the structured SCID-I/CV interview to confirm their DSM-IV schizophrenia diagnoses; therefore, it is likely that the diagnostic accuracy was good in the study.

A common problem in genetic studies is stratification bias, which can distort the results. In our study all of the patients and controls were of Finnish origin from three relatively small areas. Therefore, the sample was relatively genetically homogeneous, which is a strength of our study.

Our controls consisted of a group of randomly selected blood donors whose psychiatric status was not specifically examined. However, blood donors gave a written statement regarding their health status before every donation session. It is thus probable that blood donors represent the general population well without chronic diseases and medications.

## 6.5 Future directions

The identification of putative risk variants for schizophrenia by linkage and association studies has generated a number of replicated and non-replicated associations between genetic polymorphisms and schizophrenia. Although association studies based on individual candidate genes have suggested involvement of several genes, the results have been notably various. GWA studies have further revealed both common and rare alleles, but they have explained only a small part of the etiological variance for the disease. It seems likely that studies of CNVs and rare SNPs will also be critical in elucidating disease mechanisms. However, it is probable that common SNPs explain a larger portion of the variance than what has been determined with the existing sample sizes, with many common SNPs each having a small effect, contributing collectively to a major portion of genetic risk (Psychiatric GWAS Con-

sortium Coordinating Committee 2009). Future studies in psychiatric genetics will likely benefit from: 1) studying more homogeneous patient groups and much larger samples, 2) studying high-risk populations such as biological relatives of patients with schizophrenia, 3) using a longitudinal and prospective methodological design in order to confirm the predictive validity of neurodevelopmental clues found in patients with schizophrenia, 4) using more detailed phenotyping, 5) using better assays of genome variation, 6) genotyping at-risk subjects and combining this with functional and structural neuroimaging and neuropsychological testing in a longitudinal design, and 7) using endophenotypes (Gourion et al. 2004, Harrison 2007, Kruglyak 2008). Endophenotypes are intermediate factors between the clinical phenotype and the genotype that can be measured, but they are unseen by the unaided eye (Gottesman and Gould 2003). They are typically assessed by laboratory-based methods rather than clinical observation (Braff et al. 2007). Endophenotypes are assumed to be related more closely to relevant gene effects and involve a simpler etiological background than the disorder (Gottesman and Gould 2003, Braff et al. 2007). It has been postulated that endophenotypes are more proximal functions of gene action than is the diagnostic assignment of schizophrenia itself (Gur et al. 2007). Therefore, it should be easier to localise the genetic loci contributing to the endophenotypes than to localise those for schizophrenia (Gur et al. 2007). A disorder endophenotype should have the following features (Gottesman and Gould 2003): it should associate with the disorder in the population, be heritable and state-independent, co-segregate with the disorder, and manifest in unaffected family members of patients at a higher rate than in the general population. Suggested endophenotypic traits for schizophrenia include neuropsychological tests measuring cognitive functions (attention, verbal memory, working memory, facial processing measures) often impaired in schizophrenia (Gur et al. 2007), electrophysiological measurements of the event-related potentials P50 (Patterson et al. 2008), P300 (Bramon et al. 2004) and mismatch negativity (MMN) (Umbricht et al. 2005), eye tracking abnormalities (Kathmann et al. 2003), and physical measurements of the brain concerning, for instance, cortical grey matter and both inter- and intrahemispheric white matter (Cannon et al. 2002, Tanskanen et al. 2010). These endophenotypes have also been combined to create a diagnostic model for schizophrenia. Shan et al. (2013) studied P50 gating combined with several neuropsychological tests with 106 Chinese schizophrenia patients and 74 healthy volunteers. They used a multivariable logistic regression model to differentiate schizophrenia patients from healthy controls and the best model consisted of the covariates-impaired P50 gating,

sensitivity index from Continuous Performance Test, three indices from WAIS-III (Arithmetic, Block Design and Performance IQ), and smoking status. This model provided an excellent differentiation between schizophrenia patients and healthy controls (c statistic = 0.9043; concordant percentage = 90.4%) (Shan et al. 2013). Besides possibly helping in elucidating pathogenetic mechanisms of schizophrenia, it has been speculated that an endophenotypic approach could have therapeutic implications in the future. An example of this, discussed by Turetsky et al. (2007), is a link between familial P50 suppression deficits and α-7 subunit promoter region SNPs in the cholinergic receptor. It could be possible to identify poor P50 suppressors among patients with schizophrenia and naturally administer an α-7 agonist as adjunctive therapy or monotherapy after appropriate safety and clinical trials.

During the last three years, novel genomic approaches applied to disorders such as schizophrenia, bipolar disorder, autism spectrum disorders and attention-deficit hyperactivity disorder (ADHD) have yielded a number of important new insights. One of them is growing evidence that schizophrenia and bipolar disorder share common risk alleles (Owen 2011, Doherty et al. 2012). There is also evidence that specific CNVs that are significantly associated with schizophrenia are also associated with neurodevelopmental disorders such as autism, ADHD, intellectual disability, and epilepsy (Owen et al. 2011, Williams et al. 2010). These findings give evidence that there may be a more general overlap in genetic risk and pathophysiology among these childhood disorders and between these disorders and schizophrenia (Owen et al. 2011). It is remarkable that there are no known schizophrenia-related copy number variants for which a severe psychiatric phenotype is inevitable (Owen et al. 2011). Schizophrenia and other neurodevelopmental syndromes also have several common features, such as impairment of cognition, varying degrees of developmental delay, neurological soft signs and motor abnormalities. Because of the aforementioned findings, Owen et al. (2011) have recently suggested that these disorders could represent a continuum of genetic and environmentally induced neurodevelopmental impairment challenging the view that these are completely unrelated diagnostic entities. They propose studies examining the clinical and familial overlap between these syndromes, focusing on specific symptoms and endophenotypes and studies of relatives with less severe manifestations (Owen et al. 2011). Understanding the modifying and compensatory mechanisms that underlie variable expressivity and penetrance could help to develop new therapeutic opportunities (Owen et al. 2011).

It seems likely that the next phase of genetic studies will involve a combination of increasingly large GWAS analyses (for common SNP and copy number variation

associations) and resequencing studies (for rare variants) (Psychiatric GWAS Consortium Coordinating Committee 2009). Because hundreds of thousands to millions of SNPs are performed in GWA studies, they need a substantial correction for multiple hypothesis testing to reduce their statistical power to detect genes of a small effect. Candidate gene studies having comparatively small sample sizes and examining only a small number of polymorphisms can have relatively greater statistical power than GWA studies, in the condition that the results have been adequately tested for biological plausibility and statistical validity. Therefore, it is probable that candidate gene studies will also have a role in psychiatric genetics in the future (Cannon 2010). In addition, as more information about the pathophysiology of schizophrenia is obtained from the application of large samples to genome-wide association approaches, the application of those same large samples to detailed studies of candidate genes may lead to greater levels of success (Williams et al. 2009).

# SUMMARY AND CONCLUSIONS

Schizophrenia has been shown to be predominantly a genetic disorder with estimates of heritability of risk of around 80%. In spite of extensive research, the underlying genetic and pathophysiologic factors leading to the disorder remain unclear. Numerous linkage and candidate gene studies have revealed several regions of interest as well as evidence implicating particular genes, but no loci or genes have been impressively identified.

The aim of this thesis was to investigate the genetic basis of schizophrenia by studying the association of seven candidate genes and the risk of schizophrenia (IL-1A, IL-1B, IL-1RN, EGF, TNF-α, DRD2 and NRG-1) and the association of five candidate genes and the age of onset of schizophrenia (IL-1B, EGF, TNF-α, DRD2, NRG-1). We also tested the interaction between the IL-1B and NRG-1 genes in schizophrenia. Based on our findings and the above discussion, the following summary and conclusions can be presented.

1. The allele combination of IL-1A-889 allele 2, IL-1B-511 allele 1, and IL-1RN VNTR allele 1 was associated with the risk of schizophrenia. The risk of schizophrenia was especially heightened in subjects with IL-1A-889 allele 2, IL-1B-511 allele 1, and IL-1RN VNTR allele 1 homozygotism (I).

2. The TNF-α gene polymorphism (-G308A) was not associated with the risk of schizophrenia in the whole study population. However, the frequency of G/G homozygotes was higher in male patients with schizophrenia than in male control subjects (OR 2.00 (95% CI 1.08–3.70), P = 0.025). The TNF-α gene polymorphism was not associated with the age of onset of schizophrenia (II).

3. The EGF gene polymorphism (A61G) was associated with the age of onset of schizophrenia in the entire study population and in male subjects, but not in female subjects. The EGF gene polymorphism was not associated with the risk of schizophrenia (III).

4. The DRD2 gene polymorphism (C957T) was associated with the risk of schizophrenia, but there was no association between the DRD2 polymorphism and the age of onset of schizophrenia (IV).

5. IL-1B and NRG-1 gene polymorphisms had a significant interaction concerning the risk of schizophrenia and a trend-like interaction with the age of onset of the disease. In addition, the IL-1B gene polymorphism was associated with the age of onset of schizophrenia. No association between the NRG-1 gene polymorphism and the age of onset of schizophrenia was seen (V).

The results of this thesis give evidence that several studied genetic polymorphisms may increase susceptibility to schizophrenia and affect the age of onset of the illness. The effect of a single gene was only modest, which is in line with former studies on schizophrenia candidate genes. The strongest association was with a certain allele combination of IL-1A, IL-1B, and IL-1RN genes. Another significant finding was an interesting gene-gene interaction with the NRG-1 and IL-1B genes, which had an effect on both the risk of schizophrenia and the age of onset of the illness. The results suggest that the genes we studied act as schizophrenia susceptibility genes or modifier genes, or both. However, a future task is to replicate these findings with a larger sample, and possibly redesigning these studies to use an endophenotype approach.

In the long course of this thesis, research has taken giant leaps forward. When this study was conducted in the late 1990s and early 2000s, the principal approaches in genetic studies were genome linkage scans and candidate gene association tests. In the last few years, GWA studies with hundreds of thousands of SNPs have rev-

olutionised the genetic research and delivered many success stories in pinpointing loci for complex traits. However, a large part of the genetic variance is still unknown and a lot of work is needed to unveil the rest of the genetic influence on complex traits. In addition, the exact mechanisms with which these genetic risk polymorphisms exert their effect on the process leading to schizophrenic psychosis are largely unknown. Thus, the strategy for future studies on schizophrenia should seek to examine the measurable biological consequences of genotype/allele variation to observe their potential pathophysiological effects. Future studies will likely benefit from studying more homogeneous patient groups, studying high-risk populations such as biological relatives of patients with schizophrenia, and using a longitudinal and prospective methodological design with functional and structural neuroimaging and neuropsychological testing in order to confirm the predictive validity of neurodevelopmental clues found in patients with schizophrenia. An additional promising strategy is endophenotyping. By identifying the single SNPs associated with endophenotypes in schizophrenia, psychiatric neuroscientists can select new strong inference-based molecular targets for the treatment of schizophrenia (Braff et al. 2008). Compared to clinical psychiatric diagnoses, it is hypothesised that endophenotypes are usually simpler, more easily quantified, closer to gene expression and neural circuitry disturbances, and more amenable to gene discovery. Once the etiological factors of schizophrenia are characterised, it may be possible to develop new therapeutic opportunities for this devastating disease.

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**ORIGINAL RESEARCH ARTICLE**

# Polymorphisms of the interleukin-1 gene complex in schizophrenia

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**Keywords:** interleukin-1 gene complex; polymorphism; allele frequencies; schizophrenia

**Activation of the inflammatory response system has been related to the pathophysiology of schizophrenia by several recent studies. Schizophrenic patients have varied levels of proinflammatory cytokines, such as interleukin (IL)-1,-6, and tumor necrosis factor (TNF)α in their peripheral blood or cerebrospinal fluid.[1–6] These cytokines can modify the metabolism of neurotransmitters, influence neural development, and IL-1 has been implicated in acute, and, on the other hand, chronic neurodegeneration.[7,8] They could therefore be of primary pathogenic importance, either in the acute disease or during those stages of brain development which possibly influence the sensitivity of a person to schizophrenia in later life. The cytokine regulation of brain development and its possible neuroimmune involvement in the pathogenesis of schizophrenia has been raised.[9] One indication of the pathogenic role of IL-1 in schizophrenia would be a demonstration of the difference between schizophrenic patients and healthy controls at the gene level. Therefore we analyzed the polymorphism of the IL-1 gene complex in 50 schizophrenic patients and in 400 healthy blood donors. The following allelisms were analyzed: IL-1β gene: base exchange polymorphisms at the positions –511 (relative to the transcriptional start site); IL-1α gene: base exchange polymorphism at the position –889; IL-1 receptor antagonist (IL-1RA) gene: variable numbers of 86-base pair tandem repeats in intron 2. The frequencies of the IL-1β (–511) allele 1, IL-1α (–889) allele 2, and IL-1RA allele 1 were somewhat, but not significantly, higher in the schizophrenic patients as compared to the controls. These alleles are known to be located on the same haplotype.[10] The number of carriers of this haplotype was significantly higher in the schizophrenia patients (17/50 *vs* 81/400) than in the controls ($P = 0.026$, $\chi^2$). The frequencies of this haplotype were 0.38 and 0.27, respectively ($P = 0.0266$, $\chi^2$). The number of homozygotes of this haplotype was significantly higher in the schizophrenia patients ($P = 0.0006$, $\chi^2$). These data suggest that the cytokine aberrations in schizophrenia are, at least partly, genetically determined.**

Several studies measuring the levels of proinflammatory cytokines, such as interleukin (IL)-1, -6, and TNF-$\alpha$ in the peripheral blood or cerebrospinal fluid of schizophrenic patients have indicated dysregulation of these cytokines in schizophrenia.[1–6] We recently demonstrated that plasma IL-1$\beta$ levels were increased in a group of acute first admission drug-free schizophrenic patients as compared to healthy controls.[3] Others have linked elevated IL-6 and TNF-$\alpha$ levels to the course, the treatment or progression of schizophrenic illness.[4–6] Although these cytokine abnormalities, as well as the other immunological deviations associated with psychiatric disorders, have been known for some time, the causal relationship has remained enigmatic. Theoretically, the increase in the levels of the pro-inflammatory cytokines can simply be a consequence of mental stress or sleep deprivation associated with the onset or exacerbation of the disease,[11,12] without having a role in the pathogenesis of the disease. On the other hand, these cytokines can modify the metabolism of neurotransmitters or influence neural development.[7,8] IL-1 has been described as an astroglial growth factor and it has been suggested to have a role in the development process of the brain and to be implicated in acute and chronic neurodegeneration.[8,13] Cytokines are now known to have multiple modulatory effects on cellular growth and differentiation. These cytokines could therefore be of primary pathogenic importance, either in the acute disease phase or during those stages of brain development which possibly influence the sensitivity of a person to schizophrenia in later life. A gene-viral model of schizophrenia suggests that exposure to viruses initiates an immunological process that somehow disorganizes development of the fetal brain.[14,15]

All three genes of the IL-1 gene complex (IL-1$\alpha$, IL-1$\beta$, IL-1RA) are clustered on the long arm of human chromosome 2 in a region q13–q21.[16] The gene encoding IL-1RA has a variable number of tandem repeat polymorphisms in intron 2.[17] In the IL-1$\beta$ gene there are at least two bi-allelic base exchange polymorphisms, one at the promoter region at position –511,[18] and the other at position +3953 in the 5th exon.[19] There is now evidence that genes of Il-1$\alpha$, IL-1$\beta$, and IL-1RA, are polymorphic and the various alleles may have a differential regulatory effect on cytokine production and, consequently, the allele frequencies are often aberrant in various diseases of an autoimmune or inflammatory nature.[20,21]

We analyzed the polymorphism of the IL-1 gene complex in 50 schizophrenic patients and in 400 healthy blood donors. Our results on the IL-1 gene complex, suggest a genetic involvement in the observed dysregulation of these cytokines in schizophrenia, and thus, contrast a previous report which did not find any evidence for the association or linkage between IL-2 receptor $\beta$ chain, Il-1$\beta$, and schizophrenia.[22] The data shown in Table 1 demonstrate that distribution of the various genotypes was quite the same in schizophrenia patients as in the controls (Hardy–Weinberg equilibrium). However, the frequencies of the IL-1$\alpha$ (–889) allele 2, IL1$\beta$ (–511) allele 1 and IL-1RA allele 1 tended to be somewhat higher in the schizophrenia group. These alleles are known to locate in the same IL-1 complex haplotype.[10] The number of carriers of

**Table 1** Allele frequencies of the genes of the IL-complex in schizophrenic patients ($n = 50$) and in blood donor controls ($n = 400$)

| *Allelism (position)* | *Schizophrenic patient* | *Controls* | $\chi^2$ |
|---|---|---|---|
| IL-1$\alpha$ (–889) genotype | | | |
| 1.1 | 20 (49%) | 167 (42%) | |
| 1.2 | 21 (42%) | 201 (50%) | |
| 2.2 | 9 (18%) | 32 (8%) | |
| IL-1$\alpha$ (–889) allele frequency | | | |
| Allele 1 | 0.61 | 0.67 | $P = 0.72$ |
| Allele 2 | 0.39 | 0.33 | |
| IL-1$\beta$ (–511) genotype | | | |
| 1.1 | 25 (50%) | 146 (37%) | |
| 1.2 | 21 (42%) | 182 (46%) | |
| 2.2 | 4 (8%) | 72 (18%) | |
| IL-1$\beta$ (–511) allele frequency | | | |
| Allele 1 | 0.71 | 0.59 | $P = 0.06$ |
| Allele 2 | 0.29 | 0.41 | |
| IL-1RA (VNTR) genotype | | | |
| 1.1 | 28 (56%) | 199 (50%) | |
| 1.2 | 21 (42%) | 156 (39%) | |
| 2.2 | 1 (2%) | 37 (9%) | |
| 1.3 | 0 (0%) | 7 (2%) | |
| 2.3 | 0 (0%) | 1 (0%) | |
| IL-1RA (VNTR) allele frequency | | | |
| Allele 1 | 0.77 | 0.70 | $P = 0.66$ |
| Allele 2 | 0.23 | 0.29 | |
| Allele 3 | 0 | 0.01 | |

**Table 2** The IL-1 complex genotype in schizophrenic patients and healthy controls

Number of carriers of the IL-1$\alpha$ (–889) allele 2 pos./IL-1$\beta$ (–511) allele 2 neg./IL1RA allele 2 neg.

| | | Number | $\chi^2$ |
|---|---|---|---|
| Schizophrenia patients | ($n = 50$) | 17 (34%) | $P = 0.026$[a] |
| Controls | ($n = 400$) | 81 (20%) | |

Frequency of the IL-1$\alpha$ (–889) allele 2/IL-1$\beta$ (–511) allele 1/IL-1RA allele 1 haplotype

| | | Frequency | $\chi^2$ |
|---|---|---|---|
| Schizophrenia patients | ($n = 50$) | 0.38 | $P = 0.0266$[b] |
| Controls | ($n = 400$) | 0.27 | |

Number of IL-1$\alpha$ (–889) allele 2/IL-1$\beta$ (–511) allele 1/IL-2RA allele 1 homozygotes

| | | Number | $\chi^2$ |
|---|---|---|---|
| Schizophrenia patients | ($n = 50$) | 8 (16%) | $P = 0.0006$[c] |
| Controls | ($n = 400$) | 17 (4%) | |

[a]$\chi^2 = 4.93$, d.f. = 1, odds ratio = 2.0; [b]$\chi^2 = 4.92$, d.f. = 1; [c]$\chi^2 = 11.69$, d.f. = 1, odds ratio = 4.3.

this haplotype as well as the haplotype frequency were clearly higher in the patient group ($P = 0.026$ and $P = 0.0266$, respectively). The biological effect seems to require homozygotism as the number of IL-1$\alpha$ (–899) allele 2, IL-1$\beta$ (–511) allele 1 and IL-1RA allele 1 homozygotes was significantly higher in schizophrenic patients when compared to controls ($P = 0.0006$) (Table 2).

In conclusion, our findings on the allelism of the IL-1 gene complex suggest that the cytokine aberrations in schizophrenia are, at least partly, genetically determined. It could be speculated that the genetics of infection is somewhat different in schizophrenic patients. This could have etiopathogenic importance bearing in mind the theories of maternal viral infection leaving the foetus at greater risk of developing immunologically mediated brain changes possibly leading to schizophrenia.[15] Future studies on the genetics of immunological changes in schizophrenia should include the mothers of the patients as well.

## Methods

*Subjects*

The patient sample consisted of 50 patients (age range 19–61 years) meeting the DSM-IV criteria for schizophrenia. Twenty patients met the criteria for paranoid, nineteen for disorganized, eleven for the undifferentiated form of acute or chronic schizophrenia. Diagnoses were assigned by a Structured Clinical Interview for DSM-IV Axis I Disorders–clinician version (SCID-I/CV).[23] Patients were recruited from the Departments of Psychiatry at Helsinki University Central Hospital and at Central Hospital of South Carelia. They all gave their written informed consent for the study after its nature had been fully explained. The study was approved by the ethics committees of both hospitals.

The control group consisted of 400 healthy blood donors (age range 18–60 years, from the Finnish Red Cross Blood Transfusion Service, Tampere). Controls, as well patients, in this study were somatically healthy Caucasian Finnish citizens.

*DNA isolation and polymorphism screening*

From all subjects 10 ml of venous blood was drawn into EDTA vacuum tubes and immediately frozen at –20°C for later DNA isolation. Genomic DNA was isolated from the blood samples by using the salting-out method.[24]

Il-1Ra: A variable number of tandem repeats was analyzed as described earlier.[17] Briefly, oligonucleotides 5′CTC AGC AAC ACT CCT AT3′ and 5′TCC TGG TCT GCA GGT AA3′ were used as primers in polymerase chain reaction (PCR). Conditions used were: 96°C for 1 min followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, 70°C for 1 min, and finally 72°C for 5 min. The PCR products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide.

IL-1$\beta$ (–511): The region that contains the *Ava*I polymorphic site at the position –511 of the IL-1$\beta$ gene[18] was amplified by PCR. The oligonucleotides 5′TGG CAT TGA TCT GGT TCA TC3′ and 5′GTT TAG GAA TCT TCC CAC TT3′ flanking this region were used as primers. PCR conditions were as follows: 95°C for 2 min, 55°C for 1 min, 74°C for 1 min, then 38 cycles

of 95°C for 1 min, 55°C for 1 min, 74°C for 1 min, and finally 74°C for 4 min. The products were digested with 6 units of *Ava*I at 37°C for 3 h. Fragments were analyzed by electrophoresis on 9% polyacrylamide gel electrophoresis (PAGE), stained with ethidium bromide. This gave products of 190 bp + 114 bp (allele 1) and 304 bp (allele 2).

IL-1$\alpha$ (–889): The polymorphic region of the IL-1$\alpha$ was amplified as previously described[25] using oligonucleotide primers 5′ AAG CTT GTT CTA CCA CCT GAA CTA GGC3′ and 5′TTA CAT ATG AGC CTT CCA TG3′ based on the DNA sequences that flank the polymorphic site. Conditions used were a denaturing step of 96°C for 1 min and then 40 cycles of 94°C for 1 min, 51°C for 1 min, 72°C for 1 min, and finally 72°C for 4 min and 55°C for 5 min. The products were digested with *Nco*I and the resultant products were analyzed on 9% PAGE. This gave products of 83 bp + 16 bp (allele 1) and 99 bp (allele 2).

*Statistical analysis*

The significance of the differences of the allele frequencies in the control and patient groups was compared using the $2 \times 2$ tables and a standard $\chi^2$-test using Bonferroni correction. Statistical calculations were performed by using the Statistica software (StatSoft Inc, Tulsa, OK, USA).

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# Tumor necrosis factor-alpha −G308A polymorphism in schizophrenia in a Finnish population

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**Abstract**

Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine with functions in nerve cell growth, differentiation, and apoptosis. There are several studies showing that a TNF-α −G308A promoter polymorphism, which possibly affects TNF-α transcription, is associated with schizophrenia, although negative results also exist. Our aim was to investigate the relationship between the TNF-α −G308A promoter polymorphism, the risk of schizophrenia, and the age of onset of schizophrenia, and the TNF-α −G308A polymorphism was therefore studied in 149 southern Finnish patients with a DSM-IV diagnosis of schizophrenia and in 393 healthy controls. The allele and genotype frequencies did not differ significantly between the patient and control groups ($P = 0.10$ and 0.12, respectively), but the frequency of G/G homozygotes was statistically significantly higher in male patients than in male controls ($\chi^2 = 5.03$, d.f. = 1, $P = 0.025$) with an odds ratio of 2.00 (95% confidence interval: 1.08–3.70). No such difference was seen in female patients ($P = 0.79$) or in the whole study group ($P = 0.064$). The age of onset of schizophrenia did not differ significantly between the different TNF-α genotypes (ANOVA: $F = 0.45$, $P = 0.64$). In conclusion, we did not find a clear association between the TNF-α −G308A polymorphism and schizophrenia in the whole study group. However, TNF-α −G308A G/G homozygosity was modestly associated with schizophrenia in male patients.


*Keywords:* Tumor necrosis factor-alpha; Polymorphism; Cytokine; Schizophrenia; Association study

Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine, which is secreted in the central nervous system (CNS) by neurons, glial cells, and astrocytes [16,23,30,32,36]. TNF-α has been shown to have several functions in the CNS, including impacts on nerve cell growth, differentiation, and apoptosis. TNF-α affects the formation of neurites and neuronal survival in the CNS [26,35]. On the other hand, TNF-α has been demonstrated to have cytotoxic effects on embryonic mesencephalic dopaminergic neurons [28], which are suggested to have an important role in the etiopathogenesis of schizophrenia. The various effects of TNF-α suggest that disturbances in its levels may affect the functioning of the brain. The role of TNF-α in the pathogenesis of schizophrenia is not clear, but it has been shown to have a stimulatory effect on the catecholaminergic system, whereas chronic TNF-α release induces an inhibitory effect [43]. This is in line with the theory that acute schizophrenia involves a hyperdopaminergic state with positive psychotic symptoms, whereas chronic schizophrenia with predominantly negative symptoms is associated with a relative hypodopaminergic state [22,38]. Another piece of evidence

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suggesting that TNF-α may be implicated in the pathogenesis of schizophrenia comes from linkage studies: the TNF-α gene is located within the major histocompatibility complex (MHC) region at the short arm of chromosome 6 (6p21.1-21.3), a region that has been related to schizophrenia in several studies [12,41,48].

The TNF-α gene has several polymorphisms in the promoter region. There is a well-known biallelic base-exchange polymorphism, which consists of two alleles: a more common variant with guanine (G) (−308G) and a less common variant with adenine (A) (−308A) at position −308. This polymorphism has been shown to directly affect the transcription of TNF-α [21,25,46]. There is also evidence that the −308A allele is associated with more abundant TNF-α production [46]. However, Brinkman et al. [6] did not find any difference in the level of transcription between the −308G and −308A alleles in lipopolysaccharide-stimulated peripheral blood monocytes.

There are several recent studies concerning the possible association between the TNF-α −G308A polymorphism and schizophrenia. In the first study, Boin et al. [5] found the frequency of the −308A allele to be significantly increased in Caucasian Italian schizophrenic patients compared with controls of similar ethnic background. Genotype distribution was also significantly different between the schizophrenics and controls in their study, and −308A homozygotes were only present in the patient group. By contrast, there are two novel studies showing an increased frequency of the −308G allele in schizophrenic patients compared with controls in Caucasian German and Chinese Singaporean population groups [42,44]. Three studies have failed to show any association between the TNF-α −G308A polymorphism and schizophrenia [14,40,45]. Our recent study of 94 schizophrenic patients also failed to show any association between schizophrenia and the TNF-α −G308A polymorphism in a Finnish sample, but there was an interaction between TNF-α and the epidermal growth factor −G61A genotypes in relation to age at the onset of schizophrenia [19].

We tested the hypothesis that the TNF-α −G308A polymorphism is associated with schizophrenia in Finnish patients by studying this genetic polymorphism in a larger independent sample of 149 Caucasian Finnish schizophrenic subjects compared with 393 healthy controls. A further aim was to compare the age of onset of schizophrenic psychosis in the different TNF-α genotypes.

The patient sample consisted of 149 patients (age range 18–76 years; mean age (±S.D.) 38.7 ± 12.2 years) meeting the DSM-IV criteria for schizophrenia. There were 55 women and 94 men. Sixty-one patients met the criteria for paranoid, 47 for undifferentiated, 35 for disorganized, three for residual, and three for catatonic acute or chronic schizophrenia. The diagnoses had been assigned based on a Structured Clinical Interview for DSM-IV Axis I Disorders—clinician version (SCID-I/CV) [9] by two experienced psychiatrists (KH and HK). The exclusion criteria were: another Axis I diagnosis (substance abuse, organic mental disorders, affective disorders), neurological illness, and diabetes mellitus. The mean age (±S.D.) of onset of schizophrenic psychosis was 23.6 ± 6.3 years. We defined the age of onset of schizophrenia as the first occurrence of positive psychotic symptoms [29]. This information was acquired from the patients' medical records and from interviews with both the patients and their relatives. The mean duration (±S.D.) of schizophrenia was 15.4 ± 11.4 years. At the time of enrollment, 57 patients were being treated with clozapine, 52 patients with typical antipsychotic medication, and 31 patients with atypical antipsychotic medication other than clozapine. In nine cases the information of medication was lacking. The patients were recruited from the Departments of Psychiatry at Helsinki University Central Hospital and at South Karelia Central Hospital. All patients had been hospitalized because of acute or chronic schizophrenic psychosis. The investigators assessed the patients to be capable of understanding the study procedure after its nature had been fully explained in the interview and in a written description of the study given to them. Thereafter, all patients gave written informed consent for the study. The ethics committees of both hospitals had approved the study. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). All patients were of Caucasian Finnish origin and resident in southern Finland.

The control group consisted of 393 (213 males, 180 females) healthy blood donors (age range 19–65 years; mean age (±S.D.) 44.5 ± 11.1 years) from the Finnish Red Cross Blood Transfusion Service, Tampere, Finland. The controls were somatically healthy Caucasian Finnish citizens.

Genomic DNA was extracted from peripheral blood leukocytes of the controls using a commercially available kit (Qiagen Inc., Hilden, Germany) and from 10 ml of venous blood of the patients using the salting-out method [31]. TNF-α genotypes were determined using fluorogenic allele-specific oligonucleotide probes with the conjugated minor groove binder (MGB) group [24]. The nucleotide sequences of the primers and probes used in PCR were deduced from the published sequences deposited in the GenBank database and chosen and synthesized in conjunction with Applied Biosystems (Foster City, CA, USA), using the Assay-by-Design tool. The reporter dyes chosen were VIC (G-specific probe) and FAM (A-specific probe). DNA samples were genotyped by employing the 5′ nuclease assay for allelic discrimination using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). A PCR reaction containing genomic DNA, 1 × Universal PCR Master Mix, 900 nM of each primer, and 200 nM of each probe was performed in 96-well plates using the standard protocol for TaqMan MGB probes in a total volume of 25 μl. Water controls and known control samples previously typed by RFLP-PCR analysis were run in parallel with unknown DNA samples. After cycling, end-point fluorescence was measured and genotype calling was carried out using the allelic discrimination analysis module.

Table 1
Genotype and allele frequencies of the TNF-α −308G/A polymorphism in schizophrenic patients ($n = 149$) and healthy controls ($n = 393$)

| TNF-α −308G/A polymorphism | Schizophrenic | Controls | $\chi^2$-test *P*-value |
|---|---|---|---|
| Number of subjects | 149 (%) | 393 (%) | |
| TNF-α −308 genotype | | | |
| G/G | 122 (82) | 292 (74) | |
| G/A | 24 (16) | 95 (24) | |
| A/A | 3 (2) | 6 (2) | 0.12 |
| TNF-α −308 alleles | | | |
| Allele G | 0.90 | 0.86 | |
| Allele A | 0.10 | 0.14 | 0.10 |

The significance of the differences between the allele and genotype frequencies in the control and patient groups was determined using the $\chi^2$-test. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated to evaluate the effects of different genotypes/alleles. The differences between the genotypes concerning the continuous variables were calculated with analysis of variance (one-way ANOVA). The statistical calculations were performed by using the SPSS version 12.0 (SPSS Inc, Chicago, IL, USA) on a PC. A *P* value of less than 0.05 was considered significant. The statistical power analysis revealed that the whole sample had 50% power to detect a significant difference ($\alpha = 0.05$) between the patient and control groups. Among the males, statistical power was 61%. The power analysis was made with a power and sample size calculation program (PS program version 2.1.30).

The distributions of genotypes showed no deviation from the Hardy–Weinberg equilibrium ($P > 0.10$ in patients, and $P > 0.20$ in controls). As shown in Table 1, the distribution of genotypes was quite similar in the schizophrenic patients and the controls ($P = 0.12$). Nor were there any significant differences in the allele frequencies between the patients and the controls, although the frequency of the G allele tended to be somewhat higher in the patients than in the controls ($P = 0.10$). The frequency of TNF-α A/A homozygotes was low in both the schizophrenic and the control populations (2.0% in both groups). Because of this, we combined the A/A homozygotes and the patients with the A/G genotype in the subsequent calculations. Among the male subjects, the genotype distribution (A/A and A/G genotypes versus G/G homozygotes) between the patients and the healthy controls was significantly different ($\chi^2 = 5.03$, d.f. = 1, $P = 0.025$), with an odds ratio at 2.00 (95% CI: 1.08–3.70). No similar difference between the patients and the controls was seen in the females ($\chi^2 = 0.070$, d.f. = 1, $P = 0.79$, OR = 1.11, 95% CI: 0.52–2.34) or in the whole study group ($\chi^2 = 3.44$, d.f. = 1, $P = 0.064$, OR = 1.56, 95% CI: 0.97–2.51) (Table 2).

The age of onset of schizophrenia did not differ significantly between the different TNF-α genotypes (age of onset G/G: $23.4 \pm 1.1$ years; G/A: $24.0 \pm 2.5$ years; A/A: $26.7 \pm 1.0$ years; ANOVA: $F = 0.45$, $P = 0.64$).

Table 2
Frequency of A allele carriers in the TNF-α −308G/A polymorphism in schizophrenic patients ($n = 149$) and healthy controls ($n = 393$)

| TNF-α −308G/A polymorphism | Schizophrenic | Controls | $\chi^2$-test *P*-value |
|---|---|---|---|
| Male subjects | | | |
| A allele carriers (G/A or A/A genotype) | 16 (17%) | 62 (29%) | 0.025[a] |
| Non-A allele carriers (G/G genotype) | 78 (83%) | 151 (71%) | |
| Female subjects | | | |
| A allele carriers (G/A or A/A genotype) | 11 (20%) | 39 (22%) | 0.79[b] |
| Non-A allele carriers (G/G genotype) | 44 (80%) | 141 (78%) | |
| All subjects | | | |
| A allele carriers (G/A or A/A genotype) | 27 (18%) | 101 (26%) | 0.064[c] |
| Non-A allele carriers (G/G genotype) | 122 (82%) | 292 (74%) | |

[a] Difference between male schizophrenics and male controls: $\chi^2 = 5.03$, d.f. = 1.
[b] Difference between female schizophrenics and female controls: $\chi^2 = 0.070$, d.f. = 1.
[c] Difference between all schizophrenics and all controls: $\chi^2 = 3.44$, d.f. = 1.

Our study of 149 Caucasian Finnish schizophrenic patients and 393 healthy blood donor controls did not reveal significant differences in the genotype and allele frequencies of the TNF-α −G308A promoter polymorphism between the patients and controls in the whole patient group. This result is in line with several previous studies [14,15,19,40,45]. However, the findings on the association between the TNF-α −G308A polymorphism and schizophrenia are highly conflicting, also implying an association with both the A variant [5] and the common G variant [42,44]. Riedel et al. [40] suggested that one possible reason for these divergent results could be the ethnic differences between the different studies. It is remarkable that, in the study by Boin et al. [5], the frequency of the A allele in the Caucasian/Italian control group (0.11) was much lower than in the other published Caucasian controls (0.16–0.19) [42]. On the other hand, Tan et al. [44] reported a much higher frequency of the A allele (0.22) for their Chinese/Singaporean control group than has been previously published for Chinese controls (0.075–0.12) [10,45,49]. It remains to be clarified whether these two populations have an allele distribution different from the other Caucasian or Chinese populations. Additionally, when comparing studies with Caucasian subjects, it should be noticed that the inclusion criteria were different between our study and Boin's study [5], because the latter also included patients with schizoaffective disorder (14.3% of all patients), while we had only patients with a core diagnosis of schizophrenia. Moreover, our subjects were mostly severely ill inpatients, with a high proportion on clozapine medication (38%), which is only allowed in cases of treatment-resistant schizophrenia in Finland. It is possible that our different results compared to earlier studies indicate that the most severely ill patients, with their poor response to neuroleptic medication, may constitute

a pharmacogenetically distinct subgroup of schizophrenia. There are several studies suggesting that the patients who respond to neuroleptics and those who do not may represent two distinct groups of patients with at least partially different pathogenesis (see review by Joober et al. [18]). Recently, by combining certain genetic polymorphisms, we found a subpopulation of schizophrenia patients with a significantly higher risk of a poor response to typical neuroleptics [1]. Furthermore, the main result of the present study, namely that the TNF-α −G308A polymorphism is not associated with schizophrenia in the whole study group, is also well in line with the study of Tsai et al. [45], which included an even higher proportion of clozapine-medicated patients (48%).

In our study, the TNF-α −G308A polymorphism was not associated with the age of onset of schizophrenia. This is in line with the earlier results of Riedel et al. [40]. However, in both studies, G/G homozygotes had a lower mean age of onset than patients with the G/A or A/A genotype, but the difference was not significant.

We found TNF-α −G308A G/G homozygotism to be statistically significantly more common in our male schizophrenic patients than in the healthy male controls, whereas females displayed no differences between the patients and controls in this respect. The reason for this is unclear, but it is possible that the gender differences in estrogen levels may be one explanation for this distinction between males and females. Reduced synaptic connectivity has been suggested to have an important role in the etiopathogenesis of schizophrenia [8,11,13]. TNF-α has been demonstrated to enhance synaptic efficacy and to be required for the preservation of synaptic strength at excitatory synapses [3]. On the other hand, estrogens have also been shown to influence neural growth, induce synaptogenesis, and delay neural pruning [7,27,33,34,37,47]. The synthesis of TNF-α is regulated, in part, at the transcriptional level [4], and the TNF-α −G308A A allele has been shown to have a higher transcription rate compared with the G allele [46]. In addition, TNF-α A allele carriers produce more TNF-α in vitro than G/G homozygotes [25]. Consequently, we can speculate that the effect of the relatively lower production of TNF-α in −G308A G/G homozygotes with their possibly weakened synaptic connectivity at excitatory synapses might be compensated for in females, who have a greater estradiol effect at these synapses, reducing the risk for aberrant functioning at these neural connections.

It can be speculated that, apart from the different genotypes, genomic imprinting might also have a role in regulating TNF effects, which are possibly deviant in schizophrenia patients. Genomic imprinting refers to the silencing of one allele of a gene based on its parental origin [2]. Differential epigenetic marking of the parental chromosomes results in differential reading by the transcriptional machinery, and as a consequence, gene expression derives predominantly from one parental allele [17]. The TNF-α gene is located within the class III region of the major histocompatibility complex on chromosome 6. Rat studies have shown that there are both genomically imprinted and non-imprinted loci in MHC [20]. Thus, we cannot rule out the possibility that the TNF-α gene could be an imprinted gene. In addition, it is known that the intracellular TNF signaling pathway includes an imprinting gene Peg3/Pw1 regulating the TNF response [39]. Further study is needed to investigate genomic imprinting in the TNF signaling pathways and its potential role in the etiopathogenesis of schizophrenia.

One limitation of our study is that all of our patients were inpatients, whose psychotic symptoms were more severe than those of the average schizophrenic patient, and most of them could hence be considered treatment-resistant. Some of our results were nearly statistically significant, and we do not know whether the differences would have become significant if the study population had been larger. In addition, the low power of the study was an obvious limitation. Because of the marginal *P*-value, we cannot even rule out the possibility that the gender difference in GG homozygosity might be a coincidental finding. Furthermore, it should be noticed that this difference is explicitly due to the different genotype frequency in control males compared to male cases. Stratification bias is a common problem in studies of genetics, making them vulnerable to spurious findings. All the patients and the controls in the present study were of Finnish origin from three relatively small areas, and the sample is therefore characterized by relative genetic homogeneity.

In conclusion, we demonstrated that the TNF-α −G308A promoter polymorphism did not clearly associate with schizophrenia in our hospitalized Caucasian Finnish population. This serves as independent confirmation of our previous finding in a population of 94 Finnish schizophrenic patients [19]. However, the male subjects in the present study had a modest association between the TNF-α −G308A polymorphism and schizophrenia. Because of the limitations of the study, however, further investigations of larger samples are needed to confirm these results in other populations.

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# Epidermal growth factor a61g polymorphism is associated with the age of onset of schizophrenia in male patients

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**Abstract**

There is evidence to suggest that dysfunction of dopaminergic neurotransmission in the central nervous system (CNS) plays a role in the etiopathology of schizophrenia. Epidermal growth factor (EGF) gene polymorphism has an impact on EGF production in mononuclear cells, and EGF seems to affect the development of midbrain dopaminergic neurons. The few studies concerning EGF gene polymorphism and schizophrenia have yielded contradictory results. Our aim was to investigate whether EGF gene A61G polymorphism predisposes to schizophrenia, and this polymorphism was therefore studied in 149 schizophrenic patients and in 94 healthy controls using 5′ nucleotidase assay (TaqMan). As far as EGF A61G polymorphism was concerned, we detected no significant differences in the allele and genotype frequencies between the patients and the controls. However, the G/G genotype was significantly associated with an earlier age of onset of schizophrenic psychosis in male subjects ($P = 0.005$) as well as in the entire population, but not in female patients ($P = 0.008$ and 0.46, respectively). The average age (±SD) of onset of schizophrenia was 20.1 ± 3.9 years in male EGF A61G G/G homozygotes and 23.7 ± 6.6 ($P = 0.02$) years in other genotypes. In conclusion, EGF gene polymorphism was not associated with the risk of schizophrenia. However, the EGF G/G genotype, which has been suggested to involve abundant production of EGF, was associated with early onset of schizophrenia in male patients.


*Keywords:* Schizophrenia; EGF; Association study; Genetic polymorphism; Gender; Finnish

## 1. Introduction

The developmental model of schizophrenia suggests that the changes in the central nervous system (CNS) that sensitize a person to schizophrenia date back to the fetal period or occur later during the development of the CNS. Dopaminergic dysfunction is thought to play a role in the etiopathology of schizophrenia (Goldstein and Deutch, 1992; Kerwin, 1993; Seeman, 1993; Weinberger, 1997). Epidermal growth factor (EGF) is a polypeptide that stimulates the proliferation of ectodermal and mesodermal cells (Carpenter and Cohen, 1979). It is a member of the EGF family with structurally related factors, such as transforming growth factor (TGF) α and heparin-binding EGF-like growth factor (HB-EGF). Peptides of the EGF family exert neurotrophic and neuromodulatory effects on developing dopaminergic neurons both in vitro and in vivo (Alexi and Hefti, 1993; Casper et al., 1991, 1994; Ferrari

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et al., 1991; Plata-Salamán, 1991). Lazar and Blum (1992) demonstrated EGF production as early as at embryonic day 14 in mouse brain, and widely distributed EGF production in the brain continued to be visible up to the postnatal period in the brain, including the brainstem, cerebral cortex, hippocampus, basal hypothalamus, striatum and thalamus. These are also the brain regions where changes have been demonstrated in schizophrenia (Bogerts, 1993; Conrad and Scheibel, 1987; Shelton et al., 1988). Futamura and colleagues (2002) studied the postmortem levels of EGF family protein levels in brains of schizophrenics compared to controls and found lower EGF levels in the prefrontal cortex and striatum of schizophrenic patients. No significant differences were detected in the levels of HB-EGF and TGF α between schizophrenics and controls in any regions examined. They also found elevated EGF receptor (EGFR) expression in the prefrontal cortex in schizophrenic patients. Moreover, serum EGF levels were decreased in the peripheral blood of young drug-free patients (Futamura et al., 2002).

The EGF gene has a functional single-nucleotide polymorphism (SNP) (G–A) at position 61 (Shahbazi et al., 2002), i.e., in vitro cells from 61A homozygous individuals produce significantly less EGF than cells from G/G or A/G individuals (Shahbazi et al., 2002). The studies concerning the possible association between the polymorphism of the EGF gene and schizophrenia have thus far yielded inconsistent results (Anttila et al., 2004; Lim et al., 2005; Watanabe et al., 2005). A recent study shows that the EGF A61G and tumour necrosis factor α polymorphisms interact in early-onset schizophrenia (Kampman et al., 2004).

Because of the contradictory results of the previous studies, we conducted a case-control association study of the EGF A61G polymorphism and schizophrenia in a fully independent patient sample of 149 Caucasian Finnish schizophrenics and 94 healthy controls. Furthermore, the possible effect of the EGF gene A61G polymorphism on the age of onset of schizophrenia was studied.

## 2. Material and methods

### 2.1. Subjects

The patient sample consisted of 149 patients (age range 18–76 years; mean age (±SD) 38.9 ± 12.2 years) meeting the DSM-IV criteria for schizophrenia. There were 94 men and 55 women. Sixty-one patients met the criteria for paranoid, 46 for undifferentiated, 36 for disorganized, three for residual and three for catatonic acute or chronic schizophrenia. The diagnoses had been assigned based on a Structured Clinical Interview for DSM-IV Axis I Disorders – clinician version (SCID-I/CV) (First et al., 1997) by three experienced psychiatrists (KH, HK and JM). Exclusion criteria were: another axis I diagnosis (substance abuse, organic mental disorders, affective disorders), neurological illness and diabetes mellitus. The mean age (±SD) of onset of schizophrenic psychosis was 23.6 ± 6.3 years. We defined the age of onset of schizophrenia as the first occurrence of positive psychotic symptoms (Meltzer et al., 1997). This information was acquired from the patients' medical records and from interviews with both the patients and their relatives. The mean duration (±SD) of schizophrenia was 15.4 ± 11.3 years. We divided the patient sample into four groups according to the family background of schizophrenia with the help of the patient documents and interviews. There were 34 patients who had first-degree relatives with confirmed schizophrenia, 17 patients with schizophrenia in the extended family history (no first-degree schizophrenic relatives), 77 patients with no schizophrenic relatives and 21 patients with an undefined family background. The patients were recruited from the Departments of Psychiatry at Helsinki University Central Hospital and South Karelia Central Hospital. All the patients had been hospitalized because of acute or chronic schizophrenia. They were assessed to be capable of understanding the study procedure after its nature had been fully explained in the interview and in a written description of the study given to them. After that, all patients gave written informed consent for the study. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The ethics committees of both hospitals had approved the study. All patients were of Caucasian Finnish origin and resident in southern Finland.

The control group consisted of 94 (49 males, 45 females) healthy blood donors (age range 26–64 years; mean age (±SD) 44.9 ± 10.3 years) from the Finnish Red Cross Blood Transfusion Service, Tampere, Finland. The controls were somatically healthy Caucasian Finnish citizens.

### 2.2. EGF genotyping

Genomic DNA was extracted from peripheral blood leukocytes of the controls using a commercially available kit (Qiagen Inc., Hilden, Germany) and from patients using the salting-out method (Miller et al., 1988). Genotypes were determined with fluorogenic allele-specific oligonucleotide probes with a conjugated minor groove binder (MGB) group (Livak, 1999). The nucleotide sequences of the primers and probes used in the PCR were deduced from published sequences deposited in the GenBank database and chosen and synthesized in conjugation with Applied Biosystems (Foster City, CA, USA). DNA samples were genotyped by employing the 5′-nucleotidase assay for allelic discrimination using the ABI Prism 7000 Sequence

Detection System (Applied Biosystems, Foster City, CA, USA). PCR reaction containing genomic DNA, 1 × Universal PCR Master Mix, 900 nM of each primer and 200 nM of each probe was performed in 96-well plates using the standard protocol for TaqMan MGB probes in a total volume of 25 μl. Water controls and known control samples previously typed by RFLP-PCR analysis were run in parallel with unknown DNA samples. After cycling, end-point fluorescence was measured and genotype calling was carried out by the allelic discrimination analysis module.

### 2.3. Statistical analysis

The significance of the differences between the allele and genotype frequencies in the control and patient groups was determined using 2 × 2 tables and a standard $\chi^2$-test. The association between age at the onset of schizophrenia and EGF polymorphism was studied using the Kaplan–Meier method and the log rank test for analyses of survival. Student's *t*-test was used to compare mean ages at the onset of schizophrenia in the different EGF genotypes. A P value of less than 0.05 was considered significant. Statistical calculations were performed by using the SPSS/Win software (Version 12.0, Inc., Chicago, IL, USA).

## 3. Results

As shown in Table 1, the distribution of genotypes was quite similar in the schizophrenic patients and the controls and followed the Hardy–Weinberg equilibrium. Nor were there any significant differences in the allele frequencies between the patients and the controls, although the frequency of the G allele tended to be somewhat higher in both male and female patients than in the controls.

G allele homozygosity was significantly associated with an earlier age of onset of schizophrenic psychosis in male subjects (Kaplan–Meier log rank test $P = 0.005$) (Fig. 1). Among the male patients, the mean ages (±SD) of onset in G/G homozygotes and other genotypes were 20.1 ± 3.9 and 23.7 ± 6.6 years, respectively (Student's *t*-test $P = 0.022$). A difference in the age of onset of schizophrenia between G/G homozygotes and other genotypes was also seen in the entire patient population, but not in female subjects (Kaplan–Meier log rank test $P = 0.008$ and $P = 0.46$, respectively) (Table 2).

## 4. Discussion

### 4.1. EGF polymorphism and the risk of schizophrenia

We analyzed a G to A polymorphism at position 61 in the EGF gene in 149 schizophrenic patients and 94 healthy controls. There were no significant differences in genotype or allele frequencies between the patient and control groups, suggesting that this polymorphism does not affect the risk of schizophrenia. This is well in line with the previous studies by Lim et al. (2005) and Watanabe et al. (2005), but we could not confirm the previous finding of an association between the G allele and schizophrenia in male patients (Anttila et al., 2004).

Table 1
Allele frequencies of the gene of epidermal growth factor (EGF) in schizophrenic patients ($n = 149$) and in blood donor controls ($n = 94$)

| Gender | Allelism (position) | Schizophrenic patient | Controls | $\chi^2$-test *p*-value |
|---|---|---|---|---|
| Female | EGF(A61G) genotype | | | |
| | A/A | 17 (31%) | 18 (40 %) | |
| | A/G | 29 (53%) | 18 (40%) | |
| | G/G | 9 (16%) | 9 (20%) | |
| | EGF(A61G) allele frequency | | | |
| | Allele A | 0.57 | 0.60 | 0.70 |
| | Allele G | 0.43 | 0.40 | |
| Male | EGF(A61G) genotype | | | |
| | A/A | 39 (42%) | 20 (41%) | |
| | A/G | 36 (38%) | 22 (45%) | |
| | G/G | 19 (20%) | 7 (14%) | |
| | EGF(A61G) allele frequency | | | |
| | Allele A | 0.61 | 0.63 | 0.66 |
| | Allele G | 0.39 | 0.37 | |
| Total | EGF(A61G) genotype | | | |
| | A/A | 56 (38%) | 38 (40%) | |
| | A/G | 65 (44%) | 40 (43%) | |
| | G/G | 28 (19%) | 16 (17%) | |
| | EGF(A61G) allele frequency | | | |
| | Allele A | 0.59 | 0.62 | 0.61 |
| | Allele G | 0.41 | 0.38 | |

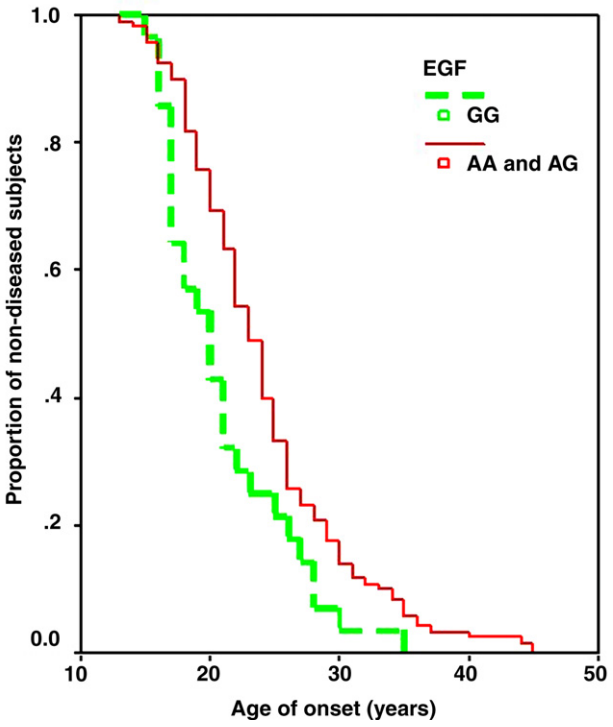


Fig. 1. Epidermal growth factor (EGF) genotypes in male schizophrenic patients and age of onset of the disease. G/G genotype ($n = 19$) vs. A/A and A/G genotypes ($n = 75$) (Kaplan–Meier, log rank test, $p = 0.005$).

Futamura and colleagues (2002) found reduced EGF levels in the peripheral blood of both medicated (45 patients and controls) and young drug-free (six patients) schizophrenics. Combined with the finding that A/A homozygotes have low levels of EGF production (Shahbazi et al., 2002), it could be suggested that A/A homozygotes would be more common among schizophrenics than controls. This is not in line with our study. One possible explanation could be that smoking reduces EGF serum levels (Ma et al., 2000), and it is well known that schizophrenics smoke more than people on an average. There are also other factors affecting serum EGF levels, such as several drugs, hormones, systemic diseases, ageing and emotional stress (Apostolakis et al., 2000; Brannvall et al., 2002; Koller et al., 2000; Pareja et al., 2003; Plata-Salamán, 1991). Furthermore, when assessing the significance of the serum EGF measurements mentioned above, it should be noticed that there were only six patients in the group of young drug-free schizophrenics (Futamura et al., 2002).

### 4.2. EGF polymorphism and age of onset of schizophrenia

Among the present male schizophrenic patients, G/G homozygotes had a significantly earlier onset of schizophrenia than A allele carriers. The average age of onset in male patients was 20.1 years for G/G homozygotes and 23.7 years for A allele carriers. This contradicts the earlier results showing the G allele to be associated with a later age of onset in male patients with schizophrenia (Anttila et al., 2004). However, it is to be noticed that the definition of age of onset was clearly different in these two studies: in the study by Anttila et al. (2004), age of onset was defined as the subject's age at the time of the first hospitalisation when the diagnosis of schizophrenia or schizophreniform psychosis was used (mean age of onset ± SD 29.6 ± 10.1 years), while in the present study, we used the definition of Meltzer et al. (1997), which equates the age at onset of schizophrenia with the first occurrence of positive psychotic symptoms (mean age of onset ± SD 23.6 ± 6.3 years). It is also possible that the controversial results could be due to differences in the patient populations: the age of onset was markedly lower in the present study than in the previous report by Anttila et al. (2004), even allowing for the different definitions of the age of onset (difference in the mean ages of onset 6.0 years). Another difference was that all of the present patients were hospitalized, most with severe psychotic symptoms, while almost half (46%) of Anttila's patients (Anttila et al., 2004) had shown a long-lasting good response to treatment with conventional neuroleptics. Because of some rather small sub-samples in our studies, a coincidental finding cannot be ruled out, either.

Table 2
Age of onset of schizophrenia according to epidermal growth factor (EGF) A61G polymorphism in male ($n = 94$) and female ($n = 55$) patients

| Gender | Genotype | Mean[a] | ±SD | Sig. ($p$-value)[b] | Δage (95% CI of the difference)[c] |
|---|---|---|---|---|---|
| Male | G/G | 20.1 | 3.9 | | |
| | Other (A/A, A/G) | 23.7 | 6.6 | 0.022 | 3.6 (0.5–6.8) |
| Female | G/G | 22.9 | 6.8 | | |
| | Other (A/A, A/G) | 24.9 | 6.0 | 0.37 | 2.0 (−2.49–6.49) |
| Total | G/G | 21.0 | 5.1 | | |
| | Other (A/A, A/G) | 24.2 | 6.4 | 0.014 | 3.1 (0.65–5.8) |

CI, Confidence interval.
[a] Years.
[b] $t$-Test (G/G vs. others).
[c] Δage, age difference between genotype groups.

Shahbazi and colleagues (2002) demonstrated that G allele carriers showed in vitro significantly more abundant production of EGF than non-carriers, and that G/G homozygotes showed higher levels of production than subjects with A/G genotype, but the latter difference did not reach statistical significance ($P = 0.097$). The peptides of the EGF family and EGFR are expressed early in fetal development (Adamson and Meek, 1984; Lazar and Blum, 1992; Nakagawa et al., 1998; Seroogy et al., 1993). EGF is known to have neurotrophic and neuromodulatory effects on neurons in the CNS (Plata-Salamán, 1991). EGF has been shown to affect the growth and survival of midbrain dopaminergic neurons, and dopaminergic dysfunction is thought to have a role in the etiology of schizophrenia (Casper et al., 1991; Casper et al., 1994; Farkas and Krieglstein, 2002; Ferrari et al., 1991; Goldstein and Deutch, 1992; Kerwin, 1993; Pezzoli et al., 1991; Seeman, 1993; Weinberger, 1997). EGF has been demonstrated to enhance dopaminergic neurotransmission by increasing dose-dependently the functional expression of D2 receptors in GH3 and GH4C1 rat pituitary tumor cells (Gardette et al., 1994; Missale et al., 1991). On the other hand, D2 receptor stimulation has been shown to enhance neurite outgrowth and branching in the CNS neurons, which means that early abnormalities in the stimulation of dopamine receptor subtypes could lead to the kinds of neuroanatomical changes observed in schizophrenia (Todd, 1992). Rats show evidence that abnormal EGF receptor stimulation during the neonatal period causes the kind of alterations in adult behavior seen in schizophrenic models (Futamura et al., 2003). Furthermore, there was a significant abnormality in dopamine metabolism in the brainstem of adult animals (Futamura et al., 2003).

EGF has also been demonstrated to reduce the release of neurotransmitter glutamate from forebrain synaptosomes (Barrie et al., 1996). Several lines of investigation have implicated glutamatergic dysfunction in the pathophysiology of schizophrenia (Goff and Coyle, 2001; Meador-Woodruff and Healy, 2000). Pharmacological studies have demonstrated that the blockade of *N*-methyl-D-aspartate (NMDA) glutamate receptors produces psychotic symptoms in normal subjects and exacerbates these symptoms in patients with schizophrenia (Lahti et al., 1995). A proposed dysfunction of glutamatergic neuronal systems is not inconsistent with the dopamine hypothesis of schizophrenia, because reciprocal synaptic relationships between forebrain dopaminergic projections and glutamatergic systems have been well described (Carlsson and Carlsson, 1990). Thus, it can be suggested that EGF G/G homozygotes producing high levels of EGF may have aberrant glutamatergic neurotransmission, which sensitizes them to the development of schizophrenic psychosis.

When all these observations are combined, we can hypothesize that the abundant EGF production in G/G homozygote schizophrenics can induce an imbalance in dopaminergic and glutamatergic neurotransmission in the brain during the fetal development of the CNS or later in life. This can be suggested to make a person more vulnerable to environmental stressors, which may contribute to the early onset of schizophrenic psychosis.

### *4.3. Gender differences in EGF polymorphism associations*

No difference in the age of onset of schizophrenia between EGF G/G homozygotes and other patients was seen in females. This gender-specific difference may result from several reasons. First, 17 β-estradiol (E2) has been shown to desensitize cells to dopamine by decreasing the number of dopamine 2 (D2) receptors in anterior pituitary cells (Pasqualini et al., 1986), and chronic estrogen treatment has been demonstrated to attenuate the functional coupling of the D2 receptor with its biochemical effector system (Munemura et al., 1989). Second, 17 β-estradiol enhances NMDA receptor phosphorylation and function (Bi et al., 2003). Overall, we can hypothesize that the higher level of estrogen production in women may attenuate hyperactive striatal dopaminergic neurotransmission and enhance glutamatergic neurotransmission in female brains and thus have a neuroprotective function against the kind of abundant EGF production seen in EGF G/G homozygotes, which means that the EGF A61G polymorphism does not affect the age of onset of schizophrenia in female subjects.

### *4.4. Limitations*

One limitation of this study is that all of our patients were inpatients, most with severe psychotic symptoms, and could hence be considered as treatment-resistant patients. The present patients had more severe symptoms than schizophrenic patients on an average. Our hypothesis of the observed association between the age of onset of schizophrenia and EGF A61G polymorphism in male schizophrenics being a consequence of different kinds of EGF production in different EGF genotypes is based on a single in vitro study with peripheral blood mononuclear cells and with rather few patients. This is also an obvious limitation in our study.

## 5. Conclusions

We demonstrated that EGF A61G polymorphism does not affect the risk of schizophrenic psychosis in general, but that there is a clear association between G-allele homozygotism in EGF polymorphism and an early age of onset of schizophrenia in male patients.

Certain limitations of this study warrant further research to confirm these findings.

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# Association between the C957T polymorphism of the dopamine D2 receptor gene and schizophrenia

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**Abstract**

The aim of this study was to investigate the relationship between the functional C957T single-nucleotide polymorphism of the dopamine D2 receptor (DRD2) gene and the risk for schizophrenia. We therefore conducted a case-control association study of 188 Finnish schizophrenia patients meeting the DSM-IV criteria and 384 healthy controls. The 5′ nuclease assay (TaqMan) was used to determine genotypes. A greater proportion of patients with schizophrenia than healthy controls were C-allele carriers (odds ratio 1.5, 95% confidence interval (CI) 1.0–2.3, $P = 0.05$). Our results are in agreement with an earlier association study suggesting that the C957T C-allele plays a role in the genetic vulnerability for schizophrenia and support the involvement of the DRD2 gene in schizophrenia pathogenesis.


*Keywords:* Association study; DRD2; Schizophrenia; Dopamine; Finnish

Schizophrenia is a common, chronic mental disorder whose symptoms typically manifest in adolescence or early adulthood. The lifetime risk of developing schizophrenia is approximately 1% in the general population [12]. Genetic factors play a major role in the disorder's etiology, and its heritability is estimated to be approximately 80% [12,14].

According to the dopamine hypothesis of schizophrenia, excessive dopaminergic activity in the mesolimbic pathway causes positive symptoms, and reduced dopaminergic activity in the mesocortical pathway causes negative symptoms and cognitive impairment [10]. *In vivo* receptor imaging studies have shown a moderate but statistically significant increase in the density of striatal postsynaptic D2 receptors in patients with schizophrenia [1,8]. Moreover, the current antipsychotic medications for schizophrenia consist of either full or partial antagonists of the dopamine D2 receptor (DRD2) [13]. Thus, the DRD2 gene is a good candidate determinant of genetic susceptibility to schizophrenia.

There are several DRD2 gene polymorphisms; the three most actively studied for a possible role in schizophrenia are the TaqI A1/A2 single-nucleotide polymorphism (SNP), the deletion of cytosine at position 141 in the promoter region (−141 Ins/Del), and the C960G missense polymorphism that leads to a Ser → Cys substitution at position 311 at the protein level (see the recent review by Dubertret et al. [4]). For the most part, previous studies investigating the association of these polymorphisms with schizophrenia have reported negative findings or have not successfully been replicated: the most consistent

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association has been found between the TaqI A1/A2 polymorphism and schizophrenia occurrence and age at schizophrenia onset [4,3].

Linkage equilibrium between the TaqI A1/A2 polymorphism and a synonymous polymorphism in the DRD2 gene, C957T, has been demonstrated [2]. *In vitro* studies have shown that the DRD2 C957T polymorphism has marked functional consequences for DRD2 mRNA stability and dopamine-regulated DRD2 expression: the T allele of this SNP is associated with decreased mRNA translation and stability, while the C allele is not [2]. In addition, Hirvonen et al. [5] have reported that C957T affects striatal D2 binding in healthy humans. Their studies suggest that striatal D2 binding is highest in T/T homozygotes, lowest in C/C homozygotes, and intermediate in C/T heterozygotes. Given that the C957T variation of the DRD2 gene plays a significant role in striatal D2 binding, it is possible that the C957T variation could be involved in the aberrant mesolimbic and mesocortical dopamine transmission in schizophrenia. In fact, in the first investigation of schizophrenia and the DRD2 C957T polymorphism, Lawford et al. [9] studied 153 schizophrenic patients and 148 controls of mixed Caucasian and Northern European origin in Australia and demonstrated an association between the C/C genotype and schizophrenia, with a population attributable risk for schizophrenia of 24%.

We report here a case-control association study of 188 schizophrenic patients and 384 healthy controls that investigated the association between the DRD2 C957T polymorphism and schizophrenia in a Caucasian Finnish population. On the basis of recent research we hypothesized that the DRD2 C957T C-allele would be overrepresented in patients with schizophrenia compared with healthy controls.

A total of 188 patients meeting the DSM-IV criteria for schizophrenia and 384 controls participated in the study. Patients were recruited from the Departments of Psychiatry at Helsinki University Central Hospital, South Karelia Central Hospital, and Tampere University Hospital and from Tampere Community Mental Health Care. An experienced psychiatrist interviewed all the patients and evaluated their hospital records to verify that they met the DSM-IV criteria. In addition, the following exclusion criteria were applied: another axis I diagnosis (substance abuse, organic mental disorder, or affective disorder), neurological illness, and diabetes mellitus. All patients were of Caucasian Finnish origin and resident in southern Finland. There were 85 women and 103 men (age 18–76 years, mean ± S.D. 43.1 ± 12.0 years). The mean age (±S.D.) at onset of schizophrenia was 27.0 ± 8.9 years, and the mean duration (±S.D.) of schizophrenia was 16.1 ± 9.6 years.

The control group consisted of 384 healthy blood donors (206 males and 178 females, age range 19–65 years, mean age ± S.D. 44.4 ± 11.2 years) from the Finnish Red Cross Blood Transfusion Service, Tampere, Finland. The controls were somatically healthy Caucasian Finnish citizens.

The study plan was approved by the local ethics committees and was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The study procedure was fully explained in an interview and a written description given to the participants. The investigators assessed all participants to be capable of understanding the study procedure, and all participants gave written informed consent to participate in the study.

Genomic DNA was extracted from peripheral blood leukocytes using a commercially available kit (Qiagen Inc., Hilden, Germany), and DNA samples were genotyped using the 5′ exonuclease assay [11]. For PCR, primers and allele-specific fluorogenic probes with conjugated minor-groove binder groups were synthesized in conjugation using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The PCR reaction mixture consisted of genomic DNA, 1× Universal PCR Master Mix, 900 nM of each primer and 200 nM of each probe. Amplification was performed using the TaqMan Universal Thermal Cycling Protocol. After PCR, end-point fluorescence intensity was measured using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), and allelic discrimination was performed. All genotyping was performed blinded to the patient outcome.

Group differences in the distribution of the DRD2 polymorphism were statistically analyzed using the Pearson chi-square test. Statistical significance was set at $P \leq 0.05$. Statistical analysis was performed using the SPSS/Win software (Version 12.0, SPSS Inc., Chicago, IL).

The genotype distributions in controls and patients followed the Hardy-Weinberg equilibrium ($P > 0.20$ and $P > 0.10$, respectively). Genotype and C957T allele frequencies for controls and patients are presented in Table 1. Analysis of the allele frequencies indicated that the C-allele was generally more common in schizophrenic patients than in healthy controls (odds ratio (OR) 1.3, 95% confidence interval (CI) 1.0–1.6; $P = 0.06$). Genotype frequencies for controls and patients did not differ from each other ($P = 0.14$). C-allele carrier status (C/T

Table 1
Genotype and allele frequencies of the DRD2 C957T polymorphism in the patient ($n = 188$) and control ($n = 384$) groups

| Group | Genotype frequency[a,b] | | | Allele frequency[c] | |
|---|---|---|---|---|---|
| | C/C | C/T | T/T | C-allele | T-allele |
| Patients ($n = 188$) | 59(31.4%) | 92 (48.9%) | 37 (19.7%) | 210(55.9%) | 166 (44.1%) |
| Controls ($n = 384$) | 104(27.1%) | 176 (45.8%) | 104 (27.1%) | 384(50.0%) | 384 (50.0%) |

[a] Genotype distribution: difference between patients and controls for C/C, C/T and T/T ($\chi^2 = 3.97$, $P = 0.14$).
[b] Genotype distribution: difference between patients and controls for C-allele carriers (C/C and C/T) vs. T/T ($\chi^2 = 3.72$, d.f. = 1, $P = 0.05$, OR = 1.5, 95%CI 1.0–2.3).
[c] Allele distribution: difference between patients and controls for C-allele and T-allele ($\chi^2 = 3.46$, $P = 0.06$, OR = 1.3, 95%CI 1.0–1.6).

and C/C) was significantly associated with risk of belonging to the patient group (OR 1.5, 95%CI 1.0–2.3; $P = 0.05$).

Our data show the DRD2 C957T C-allele to be slightly over-represented among patients with schizophrenia compared to healthy controls. In addition, genotypes with the C-allele (C/C and C/T) were more common in patients than in controls. These findings, including the reported allele distributions in patients with schizophrenia, are consistent with those of Lawford et al. [9].

Functional *in vitro* studies of the DRD2 C957T polymorphism have indicated reduced translation efficiency of DRD2 mRNA carrying the 957T mutation, leading to a 50% decrease in the level of protein synthesis compared with the C-allele [2]. Duan et al. [2] suggest that these differences may be due to changes in the predicted mRNA secondary structure. Studies in healthy human subjects have shown that the DRD2 C957T polymorphism affects striatal dopamine D2 receptor availability (binding potential) [5], with T/T homozygotes having the highest DRD2 availability and C/C homozygotes the lowest [5]. However, recent *in vivo* investigations of DRD2 density and binding affinity in healthy volunteers suggest that the C/C genotype is associated with the highest level of intrasynaptic dopamine in the striatum (lowest DRD2 affinity), the T/T genotype with the lowest level, and the C/T genotype with an intermediate level [Hirvonen et al., unpublished results]. Our data showing an increased proportion of C-allele carriers in the group of patients with schizophrenia are consistent with the aforementioned studies and the dopaminergic hypothesis of schizophrenia. They provide further support for the theory that the C-allele may predispose carriers to schizophrenia by virtue of its amplification of dopaminergic transmission, and conversely the T-allele may protect against schizophrenia by dampening dopaminergic transmission.

A limitation of the present study is that approximately two thirds of the patients were treatment-resistant, and 44% of the patients were using clozapine, which in Finland is only allowed for severe treatment-resistant schizophrenia. Hence, their symptoms were more severe than those of the average patient with schizophrenia. It is notable that in a recent study, Hwang et al. [6] investigated DRD2 C957T polymorphism and clozapine response in two Caucasian and Afro-American populations resistant or intolerant to conventional neuroleptics and found no difference between the responder and non-responder groups in either of the populations. A strength of our study is that all patients and controls were from three relatively small areas of Finland and therefore relatively genetically homogeneous. This reduces the likelihood of stratification bias, which can make genetic studies vulnerable to spurious findings. Although our results are well in line with the former study by Lawford et al. [9], it is notable that the allele distributions of the control groups in these two studies differed significantly ($P = 0.018$). The reason for this difference is unknown, but may relate to differences in ethnicity of the study participants and to the different genetic origins of Finnish Caucasians compared to Northern European Caucasians living in Australia [7]. Another reason for this difference between the two control groups might be due to the different educational status of the groups. Lawford et al.'s controls had demonstrated academic ability and hence generally excellent cognitive function, whereas our controls were voluntary blood donors that had not been selected on the basis of their educational status. It is likely that our controls were representative of the general population, including cognitive functioning.

In conclusion, our study provides support for an association between the DRD2 C957T polymorphism and schizophrenia and is consistent with the earlier findings of Lawford et al. [9] in a somewhat larger and ethnically different population. We propose that the C957T C-allele constitutes a risk factor for schizophrenia. The identification of this susceptibility locus is likely to provide valuable insights into the etiology and pathogenesis of the disorder, which may lead to the development of more effective treatments. However, given the limitations of the present study, further research with larger samples is needed to confirm these results in other populations.

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ORIGINAL PAPER

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# Interleukin-1 beta gene polymorphism and its interactions with neuregulin-1 gene polymorphism are associated with schizophrenia

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**Abstract** Interleukin-1$\beta$ (IL-1$\beta$) and neuregulin-1 (NRG-1) have an important role in development of the central nervous system. Several recent studies suggest that their genetic polymorphisms are associated with schizophrenia. We studied the effects of the IL-1$\beta$ gene (IL-1B) -511 and NRG-1 SNP8NRG221533 polymorphisms and their interactions on the risk and age of onset of schizophrenia in 113 Finnish schizophrenic patients and 393 healthy controls. The allele and genotype frequencies of IL-1B and NRG-1 did not differ between schizophrenic patients and healthy controls, but the risk of schizophrenia was more than 10 times higher (odds ratio 10.20, 95% CI 2.53–41.09, $p = 0.001$) among subjects with the IL-1B 2.2, NRG-1 CC genotypes compared to subjects with the IL-1B 2.2, NRG-1 T-allele carriage. There was also a trend for an association between the interaction between IL-1B and NRG-1 polymorphisms and the age at onset of schizophrenia ($\chi^2 = 2.80$; df = 1; $p = 0.09$, log rank test). IL-1B-511 allele 1 homozygotes had a significantly higher age of onset than allele 2 carriers (mean age of onset 25.9 ± 7.7 and 22.7 ± 5.4 years, $t$-test: $t = 2.46$; $p = 0.032$). Our results suggest that there is an interaction between the IL-1B and NRG-1 genes in schizophrenia. In addition, the IL-1B-511 polymorphism seems to be associated with the age at onset of schizophrenia.

**Key words** schizophrenia · polymorphism · interleukin-1$\beta$ · neuregulin-1 · genetics

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## Introduction

Interleukin-1beta (IL-1$\beta$) is a pleiotropic cytokine contributing to inflammation, cell growth, and tissue repair [40]. In addition, cytokines have been shown to modulate the dopaminergic neurotransmission in the central nervous system (CNS), which is thought to be aberrant in schizophrenia [29]. Numerous recent studies have provided evidence for abnormal levels of interleukin-1 (IL-1) complex cytokines in the blood of schizophrenic patients, indicating dysregulation of these cytokines in schizophrenia [1, 15, 39]. The neurodevelopmental hypothesis of schizophrenia suggests that changes in the CNS that sensitize an individual to schizophrenia are present in utero or later in CNS development. There is a good deal of evidence that IL-1$\beta$ affects CNS development. IL-1$\beta$ has been shown to stimulate astrocyte to proliferation

and produce a variety of cytokines and trophic factors, including nerve growth factor [28]. Marx et al. [23] demonstrated that IL-1$\beta$ produces dose-dependent decreases in the number of neurons in embryonic rat cortical slices, suggesting decreased cerebral cortical neuron survival. Gilmore et al. [9] showed that high levels of IL-1$\beta$ reduce dendrite development and neuron survival in vitro. These data suggest that IL-1$\beta$ plays an important role in prenatal exposure to infection and a heightened risk of schizophrenia.

The IL-1$\beta$ gene (IL-1B) has a single nucleotide polymorphism (SNP) in the promoter region at position -511 [5]. Several studies show that this SNP has an impact on the levels of IL-1 gene complex cytokines [11, 12]. We previously demonstrated an association between an allele combination of the IL-1 gene complex (i.e., IL-1alpha-889 allele 2, IL-1B-511 allele 1, IL-1 receptor antagonist (IL-1RN) variable number of tandem repeats (VNTR) allele 1) and schizophrenia, suggesting that the cytokine aberrations seen in schizophrenia might be partly genetically determined [16]. Our finding was not confirmed in two independent Chinese populations [4].

Neuregulin-1 (NRG-1) belongs to a family of neuregulins that consists of four growth factor genes that are structurally related to the epidermal growth factor genus of cell-to-cell signalling molecules [19]. NRG-1 is localized to widespread areas of the brain, including frontal cortex, hippocampus, midbrain, and cerebellum [18]. NRG-1 has a broad range of bioactivities in the CNS, including synapse formation, regulation of *N*-methyl-D-aspartate and gamma-aminobutyric acid-A receptor subunit expression, as well as neuron differentiation, proliferation, and migration [2, 20, 30, 33–35]. These activities are partly developmental, but NRG-1 continues to be expressed in the adult brain [19]. Leading theories about the pathogenesis of schizophrenia suggest a neurodevelopmental origin of the disease [24] or aberrant signalling in glutamatergic and dopaminergic pathways [3]. This makes NRG-1 an interesting candidate in the etiopathogenesis of schizophrenia.

The NRG-1 gene localizes to chromosome 8p22. It was in this region that Stefansson et al. [36] identified an NRG-1 at-risk haplotype of seven markers and one SNP in the 5′ region of the NRG-1 gene (SNP8NRG221533) that was significantly different in schizophrenic patients compared to controls in a large Icelandic sample. The same group found associations with three SNPs and the entire haplotype in a Scottish case-control sample, providing support for the initial association [37]. Williams et al. [41] examined three markers from Stefanssońs haplotype and reported an association with the haplotype but not with any of the individual markers. Yang et al. [42] investigated the single most significant SNP from the study by Stefansson et al. [36] in Chinese Han case-parent families and confirmed Stefansson's results. On the other hand, in a Finnish case-control study, the NRG-1 SNP 221533 was examined in schizophrenic patients and healthy controls, and no group differences in genotype or allele were found [14]. Nor was there any association between the polymorphism and the age of onset of schizophrenia. However, genotype distributions differed between responders and non-responders to conventional antipsychotics [14].

The purpose of the present study was to compare the allele frequencies of the IL-1B-511 polymorphism and the 8NRG221533 SNP of NRG-1 in Finnish schizophrenic patients and healthy controls and analyze the effects of these polymorphisms on the age at onset of schizophrenia. In addition, since IL-1B and NRG-1 are both candidate genes for schizophrenia and play an important role in CNS development, we studied interactions between the IL-1B-511 polymorphism and the NRG-1 SNP 221533 in the risk of schizophrenia and the age at onset of schizophrenia. The patient population in the present study was an expansion of our previous study [16] and was different from that of Kampman et al. [14].

## Methods

### Subjects

The patient sample consisted of 113 patients (69 males, 44 females; age 18–76 years, mean ± SD 39.7 ± 12.0 years) that met the DSM-IV criteria for schizophrenia. About 48 patients met the criteria for paranoid, 37 for undifferentiated, 22 for disorganized, three for residual, and three for catatonic acute or chronic schizophrenia. A family history was available for 97 patients: 24 patients had a schizophrenic first-degree relative, 13 patients had schizophrenia in more distant relatives, and 60 patients had no family history of schizophrenia. The diagnoses were assigned based on a Structured Clinical Interview for DSM-IV Axis I Disorders-clinician version (SCID-I/CV) [7] by two experienced psychiatrists (KH and HK). The following exclusion criteria were applied: an additional axis I diagnosis (substance abuse, organic mental disorder, affective disorder), neurological illness, or diabetes mellitus. The mean age ± SD of onset of schizophrenic psychosis was 23.7 ± 6.1 years. We defined the age of onset as the first occurrence of positive psychotic symptoms [26]. We acquired this information from patients' medical records and interviews with the patients and their relatives. The age of onset in one patient was unknown. The mean duration ± SD of schizophrenia was 16.3 ± 11.2 years. Patients were recruited from the Departments of Psychiatry at Helsinki University Central Hospital and South Karelia Central Hospital. All patients had been hospitalized because of acute or chronic schizophrenic psychosis. The patients were of Caucasian Finnish origin and residents of southern Finland. The control group consisted of 393 (213 males, 180 females) healthy blood donors (age range 19–65 years; mean ± SD 44.5 ± 11.1 years) from the Finnish Red Cross Blood Transfusion Service, Tampere, Finland. The controls were Caucasian Finnish citizens.

To determine interactions between IL-1B and NRG-1 polymorphisms, we divided patients and controls into four groups according to their genotype status. The groups were (1) a combination of IL-1B 1.1/1.2, NRG-1 CT/TT (frequencies in patients and controls $n$ = 87, $n$ = 287, respectively); (2) IL-1B 1.1/1.2, NRG-1 CC ($n$ = 12, $n$ = 50); (3) IL-1B 2.2, NRG-1 CT/TT ($n$ = 7, $n$ = 51); and (4) IL-1B 2.2, NRG-1 CC ($n$ = 7, $n$ = 5).

The study was approved by local hospital ethics committees and was performed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All subjects received

a full explanation and a written description of the procedures. The investigators determined that all subjects understood the procedures, and written informed consent was obtained.

### Genotyping

From each subject, 10 ml of blood was drawn into EDTA vacuum tubes and immediately frozen at −20°C. Genomic DNA was isolated from blood samples by the salting-out method [27]. Methods for screening IL-1B-511 gene polymorphisms have been described [16]. Allele assignments were made as follows: allele 1, C (cytosine); allele 2, T (thymine).

Neuregulin-1 SNP8NRG221533 was genotyped by employing the 5′ nuclease assay for allelic discrimination [21] using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). The nucleotide sequences of primers and fluorogenic allele-specific oligonucleotide probes were deduced from sequences found on the deCODE Genetics Web site (http://www.decode.com/nrg1/markers) and the GenBank Database. They were synthesized by Applied Biosystems using the Assays-by-Design tool. A PCR reaction containing genomic DNA, 1× Universal PCR Master Mix, 900 nM of each primer and 200 nM of each probe was performed in 96-well plates using the standard protocol for TaqMan (Applied Biosystems) MGB probes in a total volume of 25 µl.

### Statistical analysis

Differences between the allele and genotype frequencies in the control and patient groups were determined with a $\chi^2$ test. Differences in genotypes were calculated with *t*-tests, one-way ANOVAs, and Kaplan–Meier log rank tests. The interaction analyses were performed with a logistic regression model, and odds ratios (OR) with 95% confidence intervals (CI) were calculated with the −2 log likelihood test. Statistical significance was set at $p < 0.05$. Statistical analysis was performed using the SPSS/Win software (Version 12.0, SPSS Inc., Chicago, IL).

## Results

The genotype distributions in controls and patients followed the Hardy–Weinberg equilibrium. The distributions of IL-1B genotypes and the allele frequencies in patients and controls are shown in Table 1. There were no differences in genotype distributions between schizophrenic patients and controls ($p = 0.53$), and there were no differences in allele frequencies between the two groups ($p = 0.28$). There was no association between NRG-1 genotype or allele frequencies and schizophrenia ($p = 0.66$ and 0.36, respectively) (Table 1).

Interactions of IL-1B and NRG-1 polymorphisms were associated with the incidence of schizophrenia ($\chi^2 = 11.77$; df = 3; p = 0.008, −2 log likelihood test) (Table 2). The risk of schizophrenia was more than 10 times higher (OR 10.20, 95% CI 2.53–41.09, $p = 0.001$) for the IL-1B 2.2, NRG-1 CC-genotype combination compared to the IL-1B 2.2, NRG-1 T-allele carriage (Fig. 1).

There was no difference in the age at onset between the IL-1B-511 allele 1 carriers and 2.2 homozygotes (*t*-test: $t = 0.86$; $p = 0.39$). We also divided the patients into IL-1B-511 1.1 homozygotes and allele 2 carriers. It showed that allele 1 homozygotes had a significantly higher age of onset of schizophrenia than allele 2 carriers. The mean ± SD age of onset was 25.9 ± 7.7 years in 1.1 homozygotes and 22.7 ± 5.4 years in allele 2 carriers (*t*-test: $t = 2.46$; $p = 0.032$ (after Bonferroni correction); mean difference 3.2 years; 95% CI 0.6–5.8 years. Log rank test: $\chi^2 = 6.25$; $p = 0.012$) (Fig. 2). The NRG-1 polymorphism was not associated with the age at onset of schizophrenia ($F = 0.79$; $p = 0.46$, one-way ANOVA).

There was a trend for an association between the interaction between IL-1B and NRG-1 polymorphisms and the age at onset of schizophrenia (log rank test $\chi^2 = 2.80$; df = 1; $p = 0.09$) (Fig. 3). The mean difference in the age at onset between subgroups according to IL-1B/NRG-1 combination (subgroup I, combination of IL-1B allele 1 carriage and NRG-1 CC-genotype, $n = 11$; subgroup II, any other combination of IL-1B/NRG-1 genotypes; $n = 101$) was 4.7 years (subgroup I, mean ± SD 28.2 ± 4.2 years, subgroup II, mean ± SD 23.5 ± 6.7 years; $t = 2.2$; $p = 0.027$, *t*-test).

**Table 1** The genotype and allele frequencies of IL-1B-511 and NRG-1 polymorphisms in 113 schizophrenic patients and 393 healthy controls

| Polymorphism | Schizophrenic patients | Controls | $\chi^2$-test *p*-value* |
|---|---|---|---|
| Number of subjects | 113 (%) | 393 (%) | |
| IL-1B-511 genotype | | | |
| 1.1 | 47 (42) | 141 (36) | |
| 1.2 | 52 (46) | 196 (50) | |
| 2.2 | 14 (12) | 56 (14) | 0.53 |
| IL-1B-511 alleles | | | |
| Allele 1 | 146 (65) | 478 (61) | |
| Allele 2 | 80 (35) | 308 (39) | 0.28 |
| NRG-1 genotype | | | |
| TT | 33 (29) | 129 (33) | |
| CT | 61 (54) | 209 (53) | |
| CC | 19 (17) | 55 (14) | 0.66 |
| NRG-1 alleles | | | |
| T-allele | 127 (56) | 467 (59) | |
| C-allele | 99 (44) | 319 (41) | 0.36 |

* *p*-value refers to the difference between schizophrenic patients and controls

**Table 2** Risk of schizophrenia in relation to IL-1B and NRG-1 genotypes ($n = 113$). Genotype interaction $p = 0.008$ (−2 log likelihood test)

| Genotype combination | Odds ratio | 95% CI | *p*-value |
|---|---|---|---|
| IL-1B2.2 + NRG-1TT/CT ($n = 7$) | 1 | | |
| IL-1B1.1/1.2 + NRG-1CC ($n = 12$) | 1.74 | 0.64–4.80 | 0.28 |
| IL-1B1.1/1.2 + NRG-1TT/CT ($n = 87$) | 2.21 | 0.97–5.04 | 0.06 |
| IL-1B2.2 + NRG-1CC ($n = 7$) | 10.20 | 2.53–41.09 | 0.001 |

## Discussion

The main finding in the present study was an interaction between the IL-1B and NRG-1 genes that has implications for the genes in schizophrenia. More

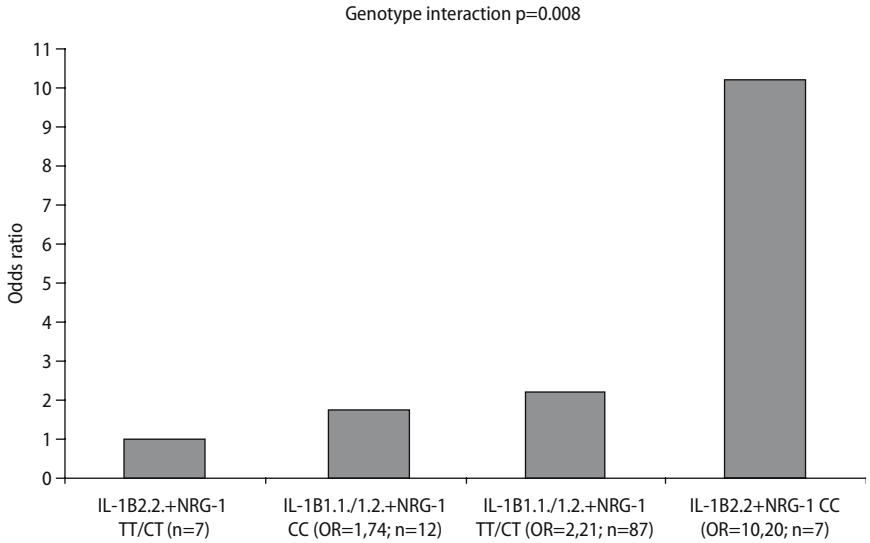


**Fig. 1** Odds ratios for having schizophrenia in relation to IL-1B and NRG-1 genotypes ($n$ = 113). Patients that were IL-1B 2.2 homozygotes and NRG-1 T-allele carriers formed a comparison group

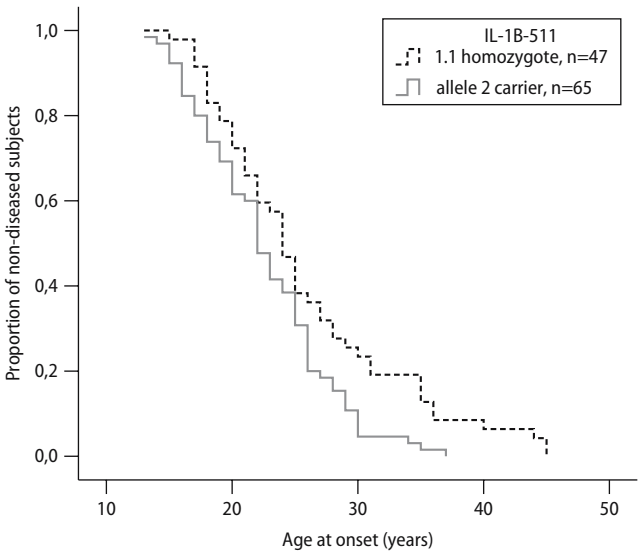


**Fig. 2** Survival plot showing the age at onset of schizophrenia in 112 patients relative to the IL-1B-511 genotype ($p$ = 0.012, log rank test)

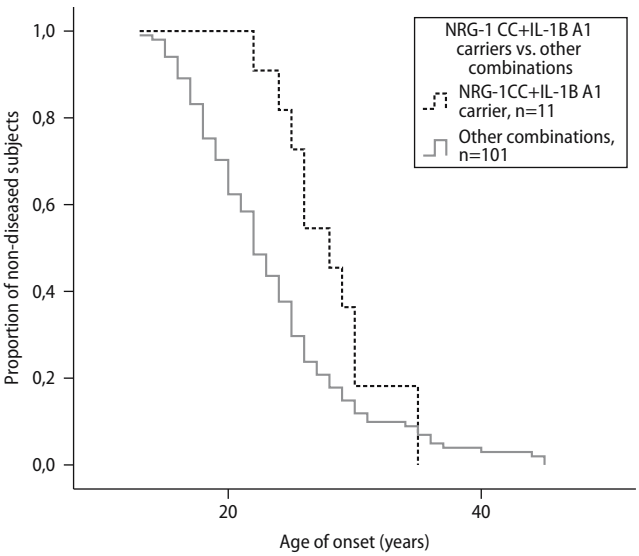


**Fig. 3** Survival plot showing the age at onset of schizophrenia in patients with the combination NRG-1 CC and IL-1B allele 1 carrier vs. all other combinations ($p$ = 0.09, log rank test) ($n$ = 112)

specifically, our results demonstrate that the interactions affect both the risk of schizophrenia and the age of onset of the disease. The interaction has not been described previously. The risk of schizophrenia was 10 times greater in patients with the IL-1B 2.2 genotype that were NRG-1 CC-homozygotes than those that were T-allele carriers. Furthermore, our results suggest that the combination of IL-1B allele 1 carriage and NRG-1 CC homozygotism is a modifier for late onset of schizophrenia, the mechanism of which is not clear. However, there is evidence that IL-1B allele 2 is associated with enhanced IL-1$\beta$ production [6, 12], and in vitro studies have demonstrated that high IL-1$\beta$ concentrations can decrease neuronal survival and may modulate the neuroanatomical alterations that are observed in schizophrenia [23]. In addition, magnetic resonance imaging (MRI) studies have demonstrated bifrontal-temporal gray matter volume deficits and generalized white matter deficits in schizophrenic IL-1B-511 allele 2 carriers [25]. We speculate that the elevated levels of IL-1$\beta$ in allele 2 carriers interact with NRG-1 in the developing brain, causing morphological and functional aberrations which might sensitize a person to a schizophrenic psychosis. Correspondingly, IL-1B allele 1 could have a protective effect for schizophrenia by delaying the onset of illness. However, it is remarkable that post-mortem studies of human dorsolateral prefrontal cortex suggest that the NRG-1 SNP 221533 appears

not to be functional, at least in terms of regulating NRG-1 expression [10].

We found no association between the IL-1B-511 polymorphism and the risk of schizophrenia. This is consistent with several previous studies [16, 17, 25, 38, 42]. However, Zanardini et al. [43] and Papiol et al. [31] reported increased IL-1B allele 1 in schizophrenia that was nearly significant. Evidence for an effect of the IL-1B-511 polymorphism on the risk of schizophrenia is weak, but haplotype analyses in the IL-1 gene complex have revealed a stronger association with schizophrenia. Zanardini et al. [43] detected a protective effect of the IL-1RN VNTR allele 2 combined with the IL-1B-511 allele 2 against schizophrenia. Papiol et al. [31] reported a significant excess of the haplotype combination of the IL-1B-511 allele 1 and the IL-RN VNTR allele 2 in schizophrenic patients compared to controls. Thus, it seems unlikely that the IL-1B-511 polymorphism is pathogenic per se, but there is evidence that its interactions with polymorphisms in other genes (e.g., other IL-1 complex genes and NRG-1) might confer an increased risk of schizophrenia.

In our study IL-1B-511 allele 1 homozygotes had a significantly higher age of onset of schizophrenia than allele 2 carriers, which suggests that this polymorphism has a modulatory effect in schizophrenia. We are not aware of any other studies that have examined the association between the IL-1B-511 polymorphism and the age at onset of schizophrenia.

There is evidence that polymorphisms of the NRG-1 gene are associated with the risk of schizophrenia [22, 36, 37, 41, 42]. In addition, Petryshen et al. [32] reported associations between a haplotype that overlaps the risk haplotype originally reported in an Icelandic population and two haplotypes located on the 3′ end of NRG-1 in a Portuguese population, but they did not detect an association with the original Icelandic risk haplotype. Fukui et al. [8] tested a four SNP haplotype, which shared three SNPs of the Stefanssoń original risk haplotype in a Japanese sample, and found a positive association. Zhao et al. [44] found an association with the risk haplotype in a Chinese Han sample. However, Iwata et al. [13] did not report a relationship in a Japanese population. Thus, the results are conflicting, and no specific mutation responsible for an association has been identified to date. The most significant association is with the SNP 8NRG221533 compared with the other six markers of the at-risk haplotype [36, 37, 42]. We did not find an association between the SNP 221533 and risk of schizophrenia in our Finnish population, which is in line with several previous studies [13, 14, 41, 44]. Nor did we find an association between the SNP 221533 and the age at onset of schizophrenia. These negative results may be due to the small sample size, which is the primary limitation of our investigation, particularly since we used the SNP 221533 as the only genetic marker, rather than a haplotype of several genetic markers.

In conclusion, we showed an interaction of IL-1B and NRG-1 polymorphisms that has an effect on the risk of schizophrenia and the age at onset of schizophrenia. In addition, we suggest that the IL-1B-511 polymorphism is a modulatory factor that affects the age of onset of schizophrenia. It is, however, important to note that the background of our findings has not been clearly verified, and is partly hypothetical. Thus, the results should be viewed cautiously. This is especially true because no gene variant in our study showed an increased risk for schizophrenia. Further studies with larger groups are needed to confirm our findings.

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