



ORIGINAL INVESTIGATION

Schizophrenia: From the brain to peripheral markers. A consensus paper of the WFSBP task force on biological markers

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Abstract

Objective. The phenotypic complexity, together with the multifarious nature of the so-called “schizophrenic psychoses”, limits our ability to form a simple and logical biologically based hypothesis for the disease group. Biological markers are defined as biochemical, physiological or anatomical traits that are specific to particular conditions. An important aim of biomarker discovery is the detection of disease correlates that can be used as diagnostic tools. **Method.** A selective review of the WFSBP Task Force on Biological Markers in schizophrenia is provided from the central nervous system to phenotypes, functional brain systems, chromosomal loci with potential genetic markers to the peripheral systems. **Results.** A number of biological measures have been proposed to be correlated with schizophrenia. At present, not a single biological trait in schizophrenia is available which achieves sufficient specificity, selectivity and is based on causal pathology and predictive validity to be recommended as diagnostic marker. **Conclusions.** With the emergence of new technologies and rigorous phenotypic subclassification the identification of genetic bases and assessment of dynamic disease related alterations will hopefully come to a new stage in the complex field of psychiatric research.

Key words: Biological markers, schizophrenia, review

Introduction

The phenotypic complexity, together with the multifarious nature of the “group of schizophrenic psy-

choses” (Bleuler 1911), limits our ability to form a simple and logical biologically based hypothesis for the disease group. With a lifetime prevalence of

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1% across the world-population and an increase in risk for children and siblings of individuals with schizophrenia of ~10 times compared to the general population there is a scientific need for validation of biological markers for the disease. In 2001, a report of the World Health Organization (WHO 2001) found schizophrenia to be the world's fourth leading cause of disability accounting for 1.1% of the total disability adjusted life years and for 2.8% of years of life lived with disability. Biomarker detection in schizophrenia has been tried for more than 20 years (Beckmann et al. 1987), with lack of useful markers in central as well as peripheral systems thus far. With the emergence of new technologies, such as gene chip-based whole genome association studies, high throughput single-nucleotide-polymorphism genotyping assays or protein profiling methods (Holmes et al. 2006; Pearson et al. 2007), the identification of genetic bases and assessment of dynamic disease related alterations will hopefully come to a new stage in the complex field of psychiatric research (Maher et al. 2008).

Biological markers are defined as biochemical, physiological or anatomical traits that are specific to particular conditions. An important aim of biomarker discovery is the detection of disease correlates that can be used as diagnostic tools. Biomarkers should furthermore have predictive power, should be available during routine assays and allow the identification of individuals at risk. Furthermore, useful markers should allow monitoring the progress of disease and treatment. At present, not a single biological trait in schizophrenia is available who achieves these guidelines. In contrast to genetically determined endophenotypes, biological markers refer to any genetically and/or environmentally determined, diagnostically valuable parameter. Biological markers can be state or trait markers, depending on the presence during certain conditions of the disease or lifelong persistence. The present paper aims to give a critical overview on our current knowledge of biomarker development in the schizophrenic psychoses.

Central nervous system

Neurotransmission

Dopaminergic neurotransmission. Since the pioneering work by Carlsson and colleagues in 1957, the role of dopamine (DA) has been extensively studied because the dopaminergic system plays a pivotal role in multidimensional brain functions, such as control and modulation of movement, cognition, memory as well as motivation and emotional behaviour. Various findings provided compelling evidence for a link between brain dopamine and schizophrenic psychoses and that the underlying abnormality of

brain functions in schizophrenia might be over-activity of dopamine mechanisms, generating the "dopamine hypothesis" of schizophrenia. Hypofrontality and attenuated DA-release in the prefrontal cortex have been proposed as pathophysiological factors in schizophrenia with a particular association with the negative symptoms of the disorder. Although hypofrontality has been proposed to be present in schizophrenia patients, there are some reports suggesting that prefrontal cortex (PFC) activity could be even higher in affected individuals when compared to normal subjects in neuropsychological tasks (Callicott et al. 2003; Manoach 2003). Thus, increases and decreases in DA release can have markedly different effects on brain function, therefore, consideration of the bi-directional nature of DA changes is important for the normal functions of brain regions receiving dopaminergic innervation including the nucleus accumbens (NAcc) and the PFC (Goto et al. 2007). The substantial neurophysiological and neuropharmacological heterogeneity shown by dopaminergic neurons originates partially from the diversity of different receptor subtypes, their anatomical localization and the immense combinatorial possibilities of neuronal connections. The anatomical organization of ascending midbrain dopaminergic neurons is complex with two distinct main nuclei in the mesencephalon, the pars compacta of the substantia nigra (SNc) forming the nigrostriatal pathway (Fuxe et al. 2006) and the ventral tegmental area (VTA) with projections to the ventral striatum as part of the mesolimbic system and to the PFC (the mesocortical system; Figure 1). Additional ascending dopaminergic connections are established with the NAcc, amygdala and hippocampus (Ferraro et al. 2007). In this circuitry the nucleus accumbens is considered as one of the main strategic interfaces for the integration of limbic signals since it receives excitatory glutamatergic projections from the PFC, the basolateral amygdala and the hippocampus. A hyperactivity of DA transmission in the mesolimbic system has, in a somewhat simplistic theory, been linked to the development of schizophrenia. Based on a relationship between the abnormal activity of dopaminergic systems and schizophrenic psychoses, the treatment with dopamine D2 receptor (DRD2) antagonists has become the main therapeutic strategy in schizophrenia. Existing anti-psychotic drugs, however, successfully control mainly the positive psychotic symptoms (delusions, hallucinations), but less the cognitive and affective deficit, despite initial hopes for the second generation antipsychotics, and do not work at all in a considerable proportion of affected individuals.

The hypothesis of increased dopamine signalling in schizophrenia originally focused on dopamine meta-

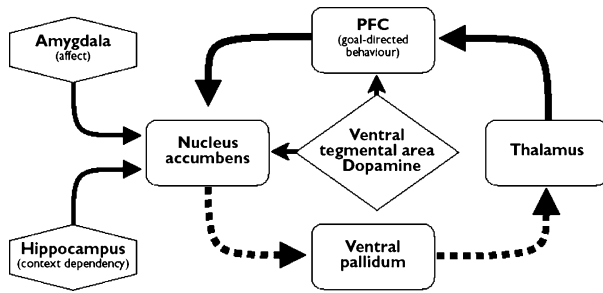


Figure 1. The proposed cortical-subcortical circuitry implicated in schizophrenia. Relevant dopaminergic, glutamatergic, and GABAergic projections are depicted with bulky, fine, and dotted arrows, respectively. The nucleus accumbens, which receives extensive dopaminergic input from the ventral tegmental area (VTA) of the midbrain and prominent excitatory input from cortical areas, the prefrontal cortex (PFC), the hippocampus, and the amygdala, is thought to play a crucial role in integrating prefrontal and limbic input. Inhibitory projections from the nucleus accumbens are sent to the ventral pallidum, which projects to specific nuclei of the thalamus, which in turn send excitatory projections to various regions of the PFC. Dopaminergic projections from the VTA also innervate the PFC. Interactions between the PFC, the amygdala, and the hippocampus are not depicted (adopted from Thompson et al. 2004).

bolism and receptor binding in the striatum with dense dopamine innervation, subsequently resulting in a macrocircuit model of a dysfunctional cortical-subcortical loop (Figure 1). On the level of the synapse, there is rather good evidence for increased, most likely presynaptic, dopaminergic transmission, whereas evidence for changes in postsynaptic DRD2 is still discussed controversial. Clinical and neuroimaging observations emphasized that the dopamine activity in the striatum is regulated by feedback from prefrontal cortex, and that striatal dopamine neuronal activity might be abnormal as a “downstream” effect of a primary prefrontal abnormality. Prefrontal dysfunction, related to abnormal prefrontal dopamine signalling, would result in disinhibition of striatal dopamine activity (Seamans and Yang 2004; Winterer and Weinberger 2004). In contrast to this hypothesis, Kellendonk and colleagues (2006) showed that overexpression of DRD2 in the striatum of D2 transgenic mice causes irreversible prefrontal deficits in motivation and working memory. Positron emission tomography (PET) imaging techniques and the development of a series of radiotracers that bind selectively to dopamine receptors or the dopamine transporter has provided new ways of assessing the functional state of the dopamine system in the human brain (Iversen and Iversen 2007). Increasing awareness of the importance of enduring negative symptoms in schizophrenia and their resistance to DRD2 antagonism has led to a reformulation of the classical dopamine hypothesis. Functional and neurochemical brain imaging studies have suggested that these symptoms might arise from altered PFC function

documenting the importance of prefrontal DA transmission at D1 receptors, the main dopamine receptor in the neocortex. The literature has, however, at the same time provided evidence for a significant role of frontal DRD2 in the development of positive symptoms (Glenthøj et al. 2006). Imaging studies documented the existence of dysregulation of striatal DA function in schizophrenia, using [^{18}F]DOPA or [^{11}C]DOPA (Table I). Several studies reported increased accumulation of DOPA in the striatum of patients with schizophrenia and high DA accumulation in patients with paranoid psychosis. Recent imaging data support the association of schizophrenia with a dopaminergic biological marker involving deficits in cortical DA function and excesses in subcortical DA function. These imaging data are consistent with the idea that both abnormalities might be secondary to a synaptic dysconnectivity involving the PFC. In turn, both components might contribute to worsen synaptic connectivity and NMDA (*N*-methyl-D-aspartate) function. Particularly in the PFC, dopamine D1 receptors were found to be up-regulated in patients with schizophrenia, whereas acute dopamine depletion studies indicated that there was an increased occupancy of DRD2 by DA at baseline in schizophrenia in comparison with healthy controls (Abi-Dargham 2004). It remains, however, unclear whether the U-curvilinear decrease in prefrontal DA innervation is an acute manifestation of illness or whether it emerges during the course of illness. DA neurotransmission also appears to affect prefrontal pyramidal neuron activity via modulation of GABA (γ -amino-butyric-acid) interneurons. Synaptic localization of DRD1 and DRD2 might further interact with the dynamics of cortical dopamine signalling in schizophrenia. Nevertheless, dopamine signalling, long considered relevant to symptomatic treatment, appears still to be a key factor in the understanding deficient functional connectivity of schizophrenia. Thus, glutamate-dopamine and dopamine-glutamate interactions and their relation to GABAergic systems might be relevant to schizophrenia pathophysiology.

Glutamatergic and GABAergic neurotransmission. Glutamate is the most important neurotransmitter in the mammalian brain and acts on different membrane-based receptor subtypes, which are divided into metabotropic and ionotropic receptors, i.e. ionotropic *N*-methyl-D-aspartate glutamate receptor (NMDAR), α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (ionotropic glutamate receptor AMPA) and kainate receptors (ionotropic kainate, glutamate receptor), and metabotropic glutamate receptors. The hypothesis of a glutamatergic hypofunction was formulated for the first time by

Table I. Imaging studies of striatal presynaptic dopamine parameters in drug-naive (DN), drug free (DF) and treated (T) patients with schizophrenia.

Parameter	Study	Number of Patients (DN/DF/T)	Radiotracer	P	Effect size
DOPA accumulation	Reith et al. 1994	5 (4/0/1)	[¹⁸ F]DOPA	<0.05	0.91
	Hietala et al. 1995	7 (7/0/0)	[¹⁸ F]DOPA	<0.05	1.54
	Dao-Castellana et al. 1997	6 (2/4/1)	[¹⁸ F]DOPA	NS	0.3
	Lindstrom et al. 1999	12 (10/2/0)	[¹¹ C]DOPA	<0.05	0.77
	Hietala et al. 1999	10 (10/0/0)	[¹⁸ F]DOPA	<0.05	1.09
	Elkashaf et al. 2000	19 (0/9/10)	[¹⁸ F]DOPA	<0.05	-0.65
	Meyer-Lindenberg et al. 2002	6 (0/6/0)	[¹⁸ F]DOPA	<0.02	1.96
	McGowan et al. 2004	16 (0/0/16)	[¹⁸ F]DOPA	0.001	1.6
Amphetamine-induced DA release	Laruelle et al. 1996	15 (2/13/0)	[¹²³ I]IBZM/amphetamine	<0.05	1.51
	Breier et al. 1997	18 (8/10/0)	[¹¹ C]raclopride amphetamine	<0.05	1.73
	Abi-Dargham et al. 1998	21 (1/20/0)	[¹²³ I]IBZM/amphetamine	<0.05	1.07
Baseline DA concentration	Abi-Dargham et al. 2000	18 (8/10/0)	[¹²³ I]IBZM/AMPT	<0.05	1.43
dopamine transporter density	Laakso et al. 2000	9 (9/0/0)	[¹⁸ F]CFT	<0.05	0.11
	Laruelle et al. 2000	22 (2/22/0)	[¹²³ I]CIT	<0.05	-0.43
	Hsiao et al. 2003	12 (12/0/0)	[^{99m} Tc]TRODAT	NS	0.22

Effect size is calculated as (mean patients – mean controls)/standard deviation controls (adopted from Toda and Abi-Dargham 2007). Abbreviations used: DOPA: dihydroxyphenylalanine; AMPT: α -methylparatyrosine; DA: dopamine; preDCA: precommissural caudate; IBZM: iodobenzamide; TRODAT is a tropane derivative.

Kim and colleagues (1980) when they showed markedly reduced cerebrospinal fluid (CSF) glutamate levels in patients with schizophrenic psychoses compared to controls. One of the main supports for the glutamatergic hypofunction is derived from the clinical effects of phencyclidine (PCP), which induces psychosis with schizophrenia-like positive and negative symptoms as well as cognitive deficits. The actions of PCP are most probably mediated via the PCP-binding site, located within the NMDAR ion channel, and depend on a high-affinity, voltage independent binding. A number of postmortem brain studies using glutamate receptor binding assays have shown either a significant increase in kainic acid and NMDA receptor binding in the frontal cortex of patients or unchanged densities of NMDA and AMPA binding sites compared to controls (Table II). Thus, expression of NMDAR appears to be variably changed in the schizophrenic psychoses (Kornhuber et al. 1989; Akbarian et al. 1996; Coyle and Tsai 2004). The same holds true for changes reported in regional post mortem brain analyses of dopaminergic D1 and D2 receptor binding densities and affinities (e.g., Seeman et al. 1984, 1987; Kornhuber et al. 1989). As a consequence the hypothesis of a regionally expressed glutamate-dopamine dysbalance has gained much interest. The glutamate hypothesis of schizophrenia is based on the assumption of the equilibrium between dopaminergic and glutamatergic neurotransmission, whereby dopamine receptor antagonists have an antipsychotic action and partial glutamate agonists possibly also have an antipsychotic effect (Figure 2).

Placebo-controlled clinical trials with agonists at the glycine-site of NMDAR have demonstrated some improvement in negative and cognitive symptoms, whereas D-serine and sarcosine reduced positive symptoms in patients receiving concurrent antipsychotics (Heresco-Levy et al. 2005; Lane et al. 2005). The thalamic filter model proposed by Carlsson (1988) marks a model, in which biochemical (glutamate, dopamine) and anatomical (frontal cortex, thalamus) hypotheses merge into a cortico-striato-thalamo-cortical control loop. A glutamatergic hypofunction is supposed to open the thalamic filter and lead to an uncontrolled flow of information to the cortex and thus to psychotic experience. This feedback loop also incorporates the action of the antipsychotic drugs that function as dopamine receptor antagonists. (Riederer et al. 1992; Mehler-Wex et al. 2006 for review). GABA-ergic interneurons play an important role in orchestrating the intermittent synchronous population-firing pattern of pyramidal neurons, which appears to be important for cortical somatosensory information processing. Many cortical GABAergic interneurons co-express and release reelin at synapses. GABAergic neurons may also coexpress parvalbumin and calretinin, however, these proteins are not secreted with GABA. The synthesis of GABA from glutamate in GABAergic neurons depends on the expression of two molecular forms of glutamic acid decarboxylase (GAD₆₇ and GAD₆₅), which are the rate-limiting enzymes in GABA biosynthesis (Akbarian and Huang 2006). Investigation of post-mortem human brain tissue from patients with schizophrenia showed alterations of cortical

Table II. Glutamate parameters measured in brain tissue of schizophrenia patients

Parameter	Method	Hippocampus	Cortex	Thalamus	Reference
NMDA receptor	Binding	▼	n.d.	▼	Kerwin et al. 1988; Kornhuber et al. 1989; Ibrahim et al. 2000
Glutamate	re-uptake	n.d.	▲	n.d.	Miyamoto et al. 2003
Glutamate	Release	n.d.	▼	n.d.	Miyamoto et al. 2003
GRIA1	Protein	▼▲	n.d.	n.d.	Eastwood et al. 1997
GRIA2/3	Protein	▼	n.d.	N.d.	Eastwood et al. 1997
NMDZ1	mRNA	▼	n.d.	▼	Gao et al. 2000; Ibrahim et al. 2000; Law and Deakin 2001
NMDE2	mRNA	▲	n.d.	▼	Gao et al. 2000; Ibrahim et al. 2000
NMDE3	mRNA	n.d.	n.d.	▼	Ibrahim et al. 2000
GRIA 1	mRNA	▼	n.d.	▼	Harrison et al. 1991; Eastwood et al. 1995; Ibrahim et al. 2000
GRIA 2	mRNA	▼		n.c.	Eastwood et al. 1995
GRIA 3	mRNA			▼	Ibrahim et al. 2000
GRIK2	mRNA	▼		▼	Ibrahim et al. 2000; Porter et al. 1997
VGluT1	mRNA	▼			Harrison et al. 2003; Eastwood and Harrison 2005
VGluT2	mRNA			▲	Smith et al. 2001a, b
EAA1	mRNA			▲	Ohnuma et al. 2000
EAA2	mRNA			▲	Ohnuma et al. 2000

Abbreviations: ▼ decreased in schizophrenia vs. controls; ▲ increased in schizophrenia vs. controls; n.c. not changed; n.d. not detected; —, not measured. NMDA, *N*-methyl-D-aspartate; GRIA, AMPA-selective glutamate receptor (synonym GluR); NMDZ1, glutamate (*N*-methyl-D-aspartate) receptor subunit 1 (synonym NR1); NMDE2, glutamate [NMDA] receptor subunit epsilon-2 (synonym NR2B); NMDE3, glutamate [NMDA] receptor subunit epsilon-3 (synonym NR2C); GRIK5, glutamate receptor, ionotropic kainate 5 (synonym KA2); VGluT, vesicular glutamate transporter; EAA, excitatory amino acid transporters (see UniProt/Swiss-Prot; pers. comm. E. Grünblatt).

GABAergic neurons, including decreases in *GAD67*, *reelin*, *GABA* membrane transporter 1 and *parvalbumin*. Consistent with a presynaptic GABAergic deficit, postsynaptic *GABRA1* function appears to be upregulated in schizophrenia. The hypothesis of dysfunctional GABAergic interneurons is supported by an increase in DNA (cytosine-5-)-methyltransferase 1 (*DNMT1*) expression in cortical and basal ganglia GABAergic neurons in schizophrenia (Veldic et al. 2007). These and earlier observations led to a hyper-

methylation hypothesis of schizophrenia (Grayson et al. 2006), where over-expression of *DNMT1* leads to promoter hypermethylation of selected genes in GABAergic neurons, finally resulting in a downregulation of multiple genes and collectively resulting in a GABAergic neuronal circuit dysfunction. The hypermethylation concept provides a coherent mechanism that establishes a link between the epigenetic misregulation hypothesis, where multiple genes collectively contribute to schizophrenia and its associated

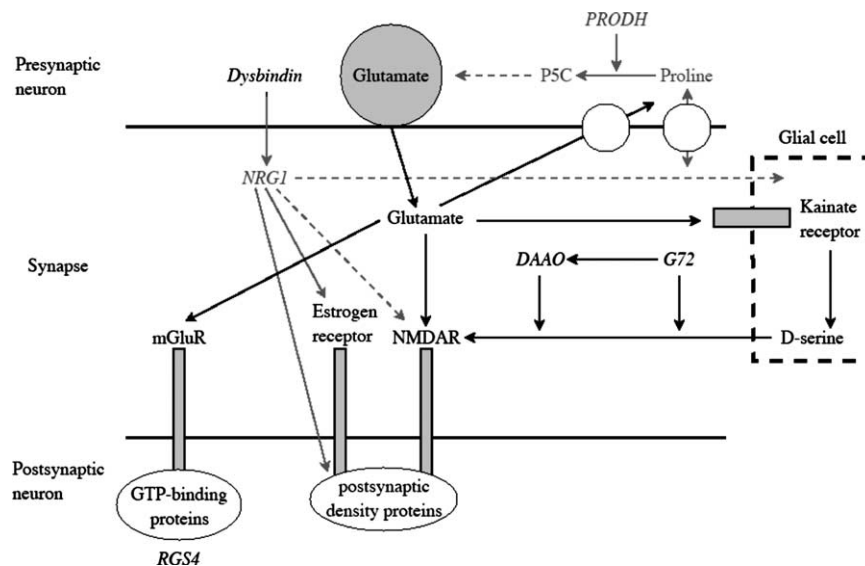


Figure 2. Schizophrenia-susceptibility genes and synaptic plasticity: The glutamatergic synapse with a hypothetical scenario in which genes (in italics) may have shared effects on synapses via influences on their formation, plasticity or signalling properties. Solid arrows point to direct interactions, dotted ones to indirect action. mGluR: metabotropic glutamate receptor, NMDA: ionotropic *N*-methyl-D-aspartate receptor, P5C: Δ^1 pyrroline-5-carboxylate; PRODH: proline dehydrogenase. (adopted from Harrison and Owen 2003).

symptomatology. According to the hypothesis of GABAergic hypofunction, GABAA receptor agonists should constitute a new class of antipsychotic drugs, administered either alone or in combination with classical antipsychotic drugs. In addition, the hypermethylation hypothesis also suggests new therapeutic targets, including the DNMT1.

Neuronal signal transduction pathways

Neuronal signal transduction pathways control neuronal positioning, neurogenesis, plasticity and integration of new neurons, whose malfunction is thought to be essential for cognition, learning, memory, and behavioural alterations in schizophrenia. Key components in signal transduction pathways and therefore candidate risk proteins in schizophrenia are reelin, DISC1 (disrupted-in-Schizophrenia 1), serine/threonine protein kinase AKT1 (V-akt murine thymoma viral oncogene homolog 1, oncogene AKT1), GSK3B (glycogen synthase kinase 3beta), and CHRNA7 (α -7-nicotinic acetylcholine receptor). Reelin (*RELN*) controls several essential steps during the embryonic phase of brain development (Herz and Chen 2006). The extracellular matrix protein reelin regulates synaptic

plasticity and protein synthesis in neuronal dendrites and their spines. Lower reelin expression levels and hypermethylation of the *RELN* promoter have been found in schizophrenia (Figure 3). *DISC1* haplotypes were reported to be associated with altered hippocampal function, including working memory and cognitive deficits in individuals with schizophrenia. *DISC1* interacts with types of phosphodiesterases (PDE4), with functional consequences for cAMP signalling, and appears to play a major role in both brain development and adult neuronal function. During brain development *DISC1* is involved in regulation of neuronal migration, neurite outgrowth, and neuronal maturation, whereas in the adult brain it modifies neuronal transmission and plasticity. AKT signalling, the most important downstream target of PI3K (phosphatidylinositol 3-kinase), is instrumental in normal dopaminergic transmission and expression of dopamine-associated behaviour. Increased apoptosis due to abnormalities in AKT signalling could contribute to the pathophysiology of schizophrenia; the PI3K-AKT pathway and GSK3 β signal transduction pathway is critical to cell growth, survival, and transcriptional regulation. In vitro studies indicated that the candidate risk protein dysbindin influences AKT signalling to

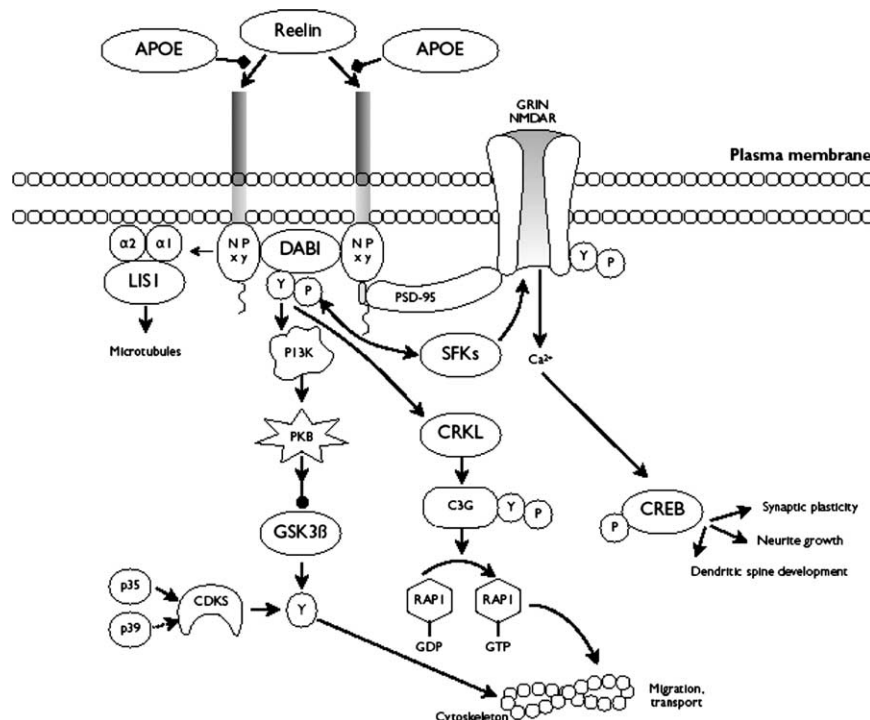


Figure 3. The reelin cascade in the aetiopathology of schizophrenia. Reelin binds to the very-low-density lipoprotein receptor (VLDLR) and APOE receptor 2 (APOER2) that results in the activation of the cytoplasmic DAB1 (adapter protein disabled 1). Phosphorylated DAB1 activates phosphatidylinositol 3-kinase (PI3K) and subsequently AKT (V-akt murine thymoma viral oncogene homologue). AKT activation inhibits the activity of glycogen synthetase kinase 3 β (GSK3 β) resulting the inhibition of τ phosphorylation that promotes microtubule stability. DAB1 also activates SRC family tyrosine kinases (SFKs) following NMDAR phosphorylation which potentiates NMDAR-mediated Ca²⁺ influx. Elevated Ca²⁺ activates cAMP-response element binding protein (CREB) that initiates expression of important genes for synaptic plasticity, neurite growth, and dendritic spine development (adopted from Herz and Chen 2006).

promote neuronal survival. A decreased AKT1 protein expression has been observed in the brains of individuals with schizophrenia, the genetic examination of different haplotypes of *AKT*, however, revealed inconsistent results regarding association with disease (Arguello and Gogos 2008; Tan et al. 2008). An additional important signalling pathway includes the acetylcholine receptors. Nicotine, the prototypical CHRNA7 agonist, is known to enhance cognition in both laboratory animals and humans; in patients with schizophrenia a reduction of CHRNA7 expression is demonstrable (Durany et al. 1999). CHRNA7 involves activation of the extracellular-signal regulated kinase (ERK) and the cAMP response element-binding protein (CREB), the signaling pathway required for cognitive processing. *CHRNA7* has been implicated in the pathogenesis of schizophrenia by genetic linkage analysis to sensory gating deficits (P50) in patients and unaffected relatives, but potential molecular variants responsible for the functional deficits await further replication (Ross et al. 2006).

Neuroimmunological markers and neurovirology

A well established finding in schizophrenia is the decreased in vitro production of interleukin-2 receptor (IL-2) and interferon- γ (IFN- γ), pointing to a blunted production of Th1 (T-helper-1) cytokines (Müller et al. 2000). Decreased levels of neopterin, a product of activated monocytes/macrophages and of the soluble intercellular adhesion molecule 1 (s-ICAM1) also represent an under-activation of the Th1 immune system (Schwarz et al. 2000). Increased serum IL-6 levels in schizophrenia are found especially in patients with an unfavourable course of the disease. IL-6 is a product of activated monocytes and of the activation of the Th2 immune response. Activation of the Th2 immune response in schizophrenic psychoses encompasses increased production of IgE (Schwarz et al. 2001), an increase of IL-10 serum levels (Cazzullo et al. 1998), and increased levels of IL-4, the key cytokine for the Th2 immune response, in the CSF of patients with juvenile schizophrenia (Mittleman et al. 1997). The decreased Th1 response and an over-activated Th2 response are associated with the enzyme indoleamine-2,3-dioxygenase (IDO), which is stimulated by Th2 cytokines. The stimulation of IDO is associated with increased production of kynurenate acid (KYNA), a product of the tryptophan/kynurenine metabolism and the only endogenous NMDAR antagonist identified so far. Thus, KYNA is able to contribute to the glutamatergic hypofunction by antagonizing NMDA, and is additionally blocking CHRNA7. The observed higher

KYNA level in the cerebrospinal fluid in critical regions of the central nervous system (CNS) on patients with schizophrenia might thus be partially an immune-mediated glutamatergic-dopaminergic dysregulation. Cyclo-oxygenase-2 (COX-2) inhibition alters Th1/Th2 cytokine balance, inducing a shift from the Th1 like to a Th2 dominated immune response. Several studies demonstrated the Th2 inducing effect of prostaglandin E₂ (PGE₂), the major product of COX-2, while inhibition of COX-2 is accompanied by inhibition of Th2 cytokines and induction of Th1 cytokines (Stolina et al. 2000). PGE₂ induces the production of IL-6, a cytokine which is increased in schizophrenia. COX-2 inhibition seems to balance the Th1/Th2 immune response by inhibition of IL-6, PGE₂, and by stimulating the Th1 immune response (Litherland et al. 1999). In a prospective, randomized, double-blind study with the COX-2 inhibitor celecoxib add-on to risperidone in acute exacerbation of schizophrenia, a therapeutic effect of celecoxib was especially pronounced regarding cognition in schizophrenia (Müller et al. 2005). The efficacy of this treatment seems most pronounced in the first years of the disease process. In view of schizophrenia as a neurodegenerative disorder activated microglial cells are observed in a subset of schizophrenia patients (Bayer et al. 1999; Radewicz et al. 2000). Microglial activation is a key factor in defending the neural parenchyma against infectious diseases, inflammation, and neurodegeneration. Risperidone significantly inhibits IFN- γ induced microglial activation in vitro, suggesting that the drug may have an anti-inflammatory effect through inhibition of microglial activation (Kato et al. 2007).

Prenatal or perinatal exposure to maternal infection is commonly accepted as a risk factor for developing schizophrenia, particularly in sporadic forms of the disease (Mednick et al. 1988; Brown et al. 2004). Less the pathogen itself, but more a pathological interaction of the pathogens with the immune response seem to be relevant to the early brain malformations found in the schizophrenic psychoses. Increased IL-8 levels in the mothers during pregnancy were associated with an increased risk for schizophrenia in the offspring (Buka et al. 2001). The link of exposure to prenatal viral infections to schizophrenia was found to be restricted to distinct phenotypes: cycloid psychoses during the first trimester and systematic schizophrenias with excess exposure to midgestational infections (Stöber et al. 1992, 1997). Persistent (chronic) infections as aetiological factor in schizophrenia are still a matter of controversy. Infections during early childhood and infections preceding the onset of illness as well as signs of inflammation in post-mortem brain studies

and antibody titres against viruses in individuals with schizophrenia have been examined for many years, however, with inconsistent results (Yolken and Torrey, 1995).

Structural brain morphology and brain morphological markers

Ultimately, the introduction of brain imaging methods has fostered the idea of schizophrenia as a brain disorder. Accordingly, there exists substantial literature on structural abnormalities based on computer tomography and in particular on structural magnetic resonance imaging studies (DeLisi 2008; Kopelman et al. 2005; Wright et al. 2000). These publications demonstrate that in schizophrenia the gray matter cortical brain volume, mainly the heteromodal association cortex such as the prefrontal cortex, is reduced, the ventricular system, especially the lateral ventricles are enlarged and temporal limbic structures such as the hippocampus are smaller with atrophy rates up to 5–10%. Prospective studies on patients with first episode schizophrenia illustrate an influence of antipsychotic medication on significant decreases in gray matter in brain volumes of patients with schizophrenia (Hulshoff et al. 2008; Lieberman et al. 2005; Okubo et al. 2001). The small amount of tissue reduction suggests that some cellular subfractions within these structures might be lost or exhibit reduced volumes. Microscopic studies of post-mortem tissue from schizophrenic patients revealed decreased neuronal or glial density and reduced neuronal size in the hippocampus. In the entorhinal cortex, abnormalities of positioning of neuronal clusters (pre- α -cells) have repeatedly been reported and support the hypothesis of migrational disturbances in schizophrenia (Falkai et al. 2000; Senitz 2008). In the PFC, the gray level index which indicates neuronal density revealed an increase in the frontal lobe suggesting circumscribed cytoarchitectonic abnormalities (Kawasak et al. 2000). Along these lines neuronal density has been reported to be increased in the PFC (Selemon et al. 2003). In layer III of this region, pyramidal neurons and other neuronal cells showed reduced somal size. A stereological study of total cell number reported decreased numbers of oligodendrocytes in layer III of the prefrontal cortex in schizophrenia (Hof et al. 2003). Taken together, it is evident that the changes in brain structure are not due to a loss of neurons but are rather a consequence of disturbed synaptic machinery leading to reduced neuropil density (dendrites and axons) in the brain regions outlined above. Looking at possible aetiological factors current risk genes and their influence on brain structures, on the macroscopic or microscopic level, are under on-going

discussion. Furthermore, environmental factors, like prenatal and perinatal noxious events, seem to have an additional effect on brain structure, mainly the hippocampus volume. A growing number of studies correspondingly examine patients at high risk for schizophrenia, indicating predictive values for the influence of structural deficits on development of psychosis (Lawrie et al. 2008).

Phenotypes of the schizophrenic psychoses

Subphenotypes

The unsatisfying search for biological markers in comparison to neighbouring medical disciplines is the result of a specific complicity of schizophrenia research. As defined in DSM-IV and ICD-10, diagnosis of schizophrenia is reduced to a minimised number of items presented through a 1–4-week period. This procedure accepts as a risk that prognostically and clinically heterogeneous disorders are lumped together, complicating comparability within and between analyzed samples and limiting the technological progress and benefits of fundamental research. Therefore splitting schizophrenia in homogeneous clinical sub-phenotypes becomes essential in genetic research and the development of biological markers (Leonhard 1999; Jablensky 2006). Linkage to 8p22-21 was reported for a phenotype with prominent affective deterioration, poorer outcome, thought disorder and fewer depressive symptoms and was referred to Kraepelin's dementia-*praecox* (Kendler et al. 2002). A study on Finnish families tested neuropsychological endophenotypes with quantitative trait markers. Positive linkage was found for the "verbal learning" subgroup on 4q13–25 and on 2q36 for the group with visual working memory deficits, with minor results such as on 15q22 for the "visual attention" group and on 9p22 for the "executive function" group (Paunio et al. 2004). In a study with Canadian families of Celtic ancestry, a susceptibility locus on chromosome 6p for the positive-symptom subgroup could be confirmed, using scores on psychopathology-symptom scales as quantitative traits. A study with Western Australian subjects used phenotypes with additional cognitive traits, defining a cognitive deficit and a cognitive spared subtype, and generated also positive results on 6p in the chromosomal region 6p25–24 for the deficit subtype (Hallmayer et al. 2005). Analyzing psychiatric diagnoses in non-schizophrenic relatives, the discrimination of co-segregating phenotypes with schizophrenia spectrum personality disorders (SSPD) and psychotic affective disorders (PAD) allowed stratifying a sample of 54 multiplex pedigrees. Two different susceptibility loci for schizo-

phrenia were detected, in the chromosomal region 8p21 for the SSPD group and on chromosome 3p26-p24 for PAD families (Pulver et al. 2000). In the differentiated concept of the endogenous psychoses (Leonhard 1999) three groups of clinical core syndromes with inherent prognostic course are proposed to form nosological entities: cycloid psychoses, unsystematic (periodic catatonia, cataphasia, affect-laden paraphrenia) and systematic schizophrenia with catatonic, hebephrenic and paraphrenic subphenotypes. A series of new research results support the nosological autonomy of these groups of illnesses (Jabs et al. 2002; Stöber et al. 2000). In the framework of international classification systems, catatonia is recognised as a cluster of gross, non-specific psychomotor traits, and mostly identifies a state of extreme motor inhibition. In view of K. Leonhard's nosological differentiation, psychomotor disturbances are complex, and as a basic point quantitative hyperkinetic or akinetic changes (motility psychoses with phasic remitting course) have to be discriminated from qualitative changes, true "catatonic" signs. Periodic catatonia is a bipolar disorder in the schizophrenic spectrum with prominence of qualitative psychomotor changes. Two psychotic poles, psychomotor excitement and inhibition, involve parakinesia, grimacing or mask-like facies, iterations, posture stereotypies, as well as distorted stiff movements, or akinetic negativism. In most cases, acute psychotic episodes are accompanied by hallucinations and delusions. In remission, there remains a distinct mild to severe catatonic residual state with psychomotor weakness and diminished incentive. Gjessing's concept of periodic catatonia pooled bipolar psychomotor disorders with phasic course and those with episodes of worsening. In periodic catatonia, prevalence is ~1:10,000 in the general population, within the affected families the morbidity risk is ~27% for first-degree relatives, with an estimated penetrance of the disorder of ~40% (Leonhard 1999; Beckmann et al. 1996; Stöber et al. 1995, 2000). Periodic catatonia is the first sub-phenotype of the schizophrenic psychoses with confirmed linkage despite considerable genetic heterogeneity in two independent genomewide linkage scans (Stöber et al. 2000, 2002). Major disease loci, supported by independent pedigrees, were observed at chromosome 15q and 22q. In catatonia, systemic pathophysiology and the involved neuro-anatomical structures remain still undetermined, but basal ganglia and thalamo-cortical loops seem to be involved (Lauer et al. 2001). Using broadly defined criteria for catatonia, imaging techniques revealed during acute akinesia a decrease of blood flow in right lower and middle prefrontal and parietal cortex, motor activation was reduced in the contralateral

motor cortex, and in a single case study acute akinesia caused a reversible complex dysregulation of glucose metabolism in large brain areas (De Tiège et al. 2003; Northoff 2000). A multifactorial aetiology is suggested in the pathology of the phasic cycloid psychoses with preponderance of non-genetic factors. Concordance rate for monozygotic pairs (39%) and for dizygotic pairs (31%) reflect a low heritability (Franzek and Beckmann 1998), the cumulative morbidity risk of first-degree relatives in index cases with cycloid psychoses compared to manic-depressive illness, and community controls generated a morbidity risk of 10.8% (cycloid psychosis), 35.2% (manic-depression) and 5.7% (control group), if bipolar disorders are rigorously defined (Pfulmann et al. 2004). Among the somatic factors, early exposure to gestational infections (first trimester) and other obstetric complications are worth mentioning (Stöber 2005). Studies of event-related potentials revealed distinct characteristics in cycloid psychoses, but there have yet been only two neuroimaging studies explicitly investigating functional correlates of cycloid psychoses, suggesting a global "hyperactivity" during the phasic occurrence of cycloid psychoses favouring occipital on cost of frontal perfusion, particularly on the right side of the brain, whereas "acute hyperfrontality" is redistributed to occipito-temporo-parietal areas upon their treatment and remission (Strik et al., 1996; Jabs et al. 2002). The systematic schizophrenias are the most debilitating schizophrenic diseases with distinct clinical pictures pointing to specific circumscribed functional psychic fields, so that in each subtype a definite higher function of the nervous system is involved. Supporting the prenatal viral infection theory as a part of the neurodevelopment concept of schizophrenia, second trimester infections, in particular during the fifth month of gestation, were significant related to the development of systematic schizophrenias with chronic non-remitting course and characteristic residual syndromes (Stöber et al. 2005). In these disease phenotypes, 28% were exposed to severe midgestational infections. In the subcategory of systematic catatonias, the risk of being exposed reached odds ratio (OR) = 23.33 (95% CI 11.7–273.4). Post-mortem investigations on the brains of schizophrenics have shown evidence for circumscribed malformations, nerve cell alterations as well as cytoarchitectural deviations attributable to disruptions of neural migration in the second trimester of gestation (Jakob and Beckmann 1986; Beckmann 2001). These findings are suggestive that viral infections may result in selective destructions or imbalances of neuronal pathways in the foetal brain. An attractive hypothesis in the aetiology of systematic catatonia is the affection of developing inhibitory

circuits with disinhibition of GABAergic and glutamatergic neurotransmission with consecutive excitotoxicity and progressive disease course. For SNPs and haplotypes in the GABAA receptor b2 subunit gene (GABRB2) significant associations were observed with systematic schizophrenia (mainly with combinations of tSNPs rs10060148, rs1816072 and rs1816071) compared to control populations (Lo et al. 2007). Regarding neurophysiological markers the dual click P50 paradigm (abnormal sensory gating) seems to discriminate systematic schizophrenias with prominent negative symptoms and without perceptual abnormalities from cycloid psychoses and healthy volunteers (Ringel et al. 2004).

Endophenotypes

Endophenotypes are objectively measurable, biologically anchored heritable traits, which should co-segregate with clinical illness in pedigrees and may also be expressed in clinically unaffected family members. The present interest in endophenotypes reflects a degree of dissatisfaction with the limited success of genetic studies of psychiatric disorders defined on the basis of DSM and ICD. There is an overall agreement that phenotypic variation and pathogenetic heterogeneity is not adequately captured by current clinical classifications. Table III summarizes the features of promising endophenotype approaches, including measures of cognition, electrophysiological brain responses, and brain imaging techniques. Application of the endophenotype approach to parsing the complexity of the disorder is likely to facilitate its genetic analysis. Psychophysiological measures that seem to fulfil the criteria of candidate endophenotypic markers for schizophrenia include prepulse inhibition of the startle reflex (PPI), P50 suppression, P300 amplitude and antisaccade eye movements (Calkins et al. 2007; Turetsky et al. 2007). The startle reflex is the central feature in PPI research. The reflex is elicited by a sudden and intense stimulus, usually acoustic in nature, and is usually assessed by electromyographic recordings of the orbicularis oculi muscle. In healthy subjects the reflex is attenuated if the startle eliciting stimulus is preceded by a less-intense stimulus, the so-called prepulse inhibition (Braff et al. 2001). The P50 event related potential (ERP) is a positive electroencephalographic (EEG) potential measured approximately 50 ms after a usually acoustic stimulus is presented to a subject. In healthy individuals, the P50 amplitude to the second of the two identical stimuli is usually suppressed compared to the first stimulus. A P300 amplitude is elicited by infrequent stimuli (deviants) appearing in a sequence of frequent stimuli (standards). Maximum P300 ampli-

tude is commonly observed, when the subject is requested to respond to these deviant stimuli, e.g. by pressing a button. The P300 is differentiated in a non-attentive, fronto-centrally located P3a subcomponent and an attentive more posterior located P3b subcomponent (Polich and Criado 2006). In a typical antisaccade eye-movements task, a subject is asked to fixate a visual stimulus on a monitor, followed by presentation of an unpredictably located stimulus to the left or right. The subject is requested to look in the mirror image location on the opposite site of the screen, thereby inhibiting an unwanted reflexive movement of the eyes towards the stimulus (Everling and Fischer 1998). An additional major advantage of all of the above mentioned candidate psychophysiological endophenotypic markers for schizophrenia is that they are reasonably easy to assess, i.e. with a minimum cooperation of the subjects and in a reasonable amount of time. A further benefit is that there is a vast amount of literature in which patients with schizophrenia show deficits in these four psychophysiological phenomena, since they each have been studied for more than three decades. Furthermore, the auditory N100 amplitude seems a heritable trait that is abnormal in patients and a subset of relatives for whom psychiatric comorbidity may be a genetically associated phenotype (Turetsky et al. 2008). Future research should focus on other promising candidates for psychophysiological markers of schizophrenia such as the N100 amplitude, processing negativity, mismatch negativity and possibly gamma band activity, and link the psychophysiological endophenotypes to each other.

Neuromotor markers and minor physical anomalies

The currently most dominant theory for the development of schizophrenia is the “neurodevelopmental theory” (Conrad and Scheibel 1987; Jakob and Beckmann 1986). The conceptual ground of this theory was laid by Barbara Fish in the early 1950s, and basically postulates that schizophrenia is a somatic brain disease caused in part by disturbances in early neurodevelopment, occurring during cell development, integration, pruning, etc., and resulting from both genetic and environmental influences. This leads to vulnerability that interacts with other influences during the continuing process of CNS maturation, sometimes resulting in clinically manifest schizophrenia. As the exact influences and their modes of (inter) action were not specified, this more represents a “model” than a specific “theory”. Subsequent versions have elaborated on the presence and timing of later influences, in attempts to integrate new knowledge of the continuing change-

Table III. Synopsis of selected characteristics of candidate endophenotypes in schizophrenia research (pers. comm. A. Jablensky)

Domain/Tests	Association with disorder	Effect size	Trait stability/State independence	Heritability/ Segregation within Families	Underlying neurobiology /genetics
a) Cognition					
<i>Attention</i> Continuous Performance Task (CPT) Identical Pairs (CPT-IP): working memory load Degraded Stimulus (CPT-DS): perceptual disambiguation	SZ patients consistently worse than controls. CPT-IP associated with negative symptoms; CPT-DS associated with cognitive disorganization	CPT-IP $d = 1.51$ CPT-DS $d = 1.29$ (WAFSS)	Test-retest: CPT-IP $r = 0.56$ (SZ) 0.73 (C) CPT-DS $r = 0.65$ (SZ) 0.72 (C) Both stable across clinical states	Twin studies: CPT-IP $h^2 = 0.39-0.49$ (C) CPT-DS $h^2 = 0.51-0.57$ (C) CPT-DS $\lambda_r = 9$ (sibs) $\lambda_r = 12$ (parents)	Linkage to 6p24 (WAFSS). Association with the 22q11 deletion syndrome
<i>Verbal declarative memory (VDM)</i> WMS-III LM CVLT RAVLT	The most consistently found deficit in SZ patients. VDM deficits related to negative symptoms	$d = 1.41-2.39$	Test-retest: $r = 0.62-0.64$ (SZ) $r = 0.74-0.88$ (C)	Twin studies: $h^2 = 0.47-0.63$ (C) $h^2 = 0.21-0.49$ (SZ) Mild deficits in unaffected family members.	VDM deficits associated with decreased volume of hippocampus; association with DISC1 reported.
<i>Working memory (WM)</i> Online maintenance of information: Spatial delayed response Digits forward Maintenance + manipulation N-back tasks Digits backward Letter-number sequencing (LNS)	Consistently found deficits in SZ patients	LNS > 1.4 SD (SZ vs controls) LNS $d = 0.66$ (unaffected family members vs controls)	Constancy over time; Minimal correlation with positive symptoms; moderate correlation with negative symptoms.	Twin studies: $h^2 = 0.43-0.49$ (C) $h^2 = 0.36-0.42$ (SZ)	WM deficits associated with DLPFC and posterior parietal dysfunction. Likely role of COMT rs 4680 (Val ^{158/108} Met) and DISC1.
<i>Face memory and emotion processing</i> Face recognition tasks Emotion recognition tasks	Frequently found deficits in SZ patients	No published data	Possibly stable trait; more data required.	Twin studies: $h^2 = 0.33$ (faces) $h^2 = 0.37$ (emotion) Mild deficits in unaffected family members	Associations (fMRI): Faces: right fusiform gyrus and frontotemporal circuitry. Emotion: amygdala and hippocampus. Genetic association not known.
b) Neurophysiology/psychophysiology					
<i>Sensory gating</i> P50 event-related potential (suppression ratio and amplitude difference)	SZ patients fail to attenuate response to second (test) stimulus.	Amplitude: $d = 0.78$ Suppression ratio: $d = 0.54$ (WAFSS)	Test-retest: $r = 0.66-0.73$ Suppression deficit present in both acutely psychotic and stabilized patients. Clozapine reduces deficit.	Twin studies: $h^2 = 0.53-0.68$ Unaffected relatives and high-risk subjects show less suppression than controls, but data are inconsistent.	Cholinergic activation of hippocampal CA3/CA4 interneurons inhibits the firing of pyramidal neurons. Involvement of temporoparietal and prefrontal circuits likely. Association with <i>CHRNA7</i> .

Table III (Continued)

Domain/Tests	Association with disorder	Effect size	Trait stability/State independence	Heritability/ Segregation within Families	Underlying neurobiology /genetics
<i>Failure of automatic inhibition</i> Prepulse inhibition of startle reflex (PPI)	SZ patients are deficient in automatic inhibition of startle. The response is influenced by atypical antipsychotics and nicotine. DA agonists reduce, NMDA antagonists increase PPI.	Not available	Test-retest: $r > 0.90$ Longitudinal stability of PPI across clinical states has been little investigated.	Twin studies: $h^2 > 0.50$ PPI deficits found in unaffected family members. Males produce greater PPI than females. PPI in Asians > Caucasians	PPI regulated by a limbic cortico-striato-pallido-pontine circuit interacting with the primary startle circuit at the pons reticular nucleus. PPI is abnormal in the 22q11 deletion syndrome. Possible role of the D2-like receptor G-protein and hippocampal $\alpha 5$ subunit of the GABA _A receptor.
<i>Stimulus deviance detection</i> Mismatch negativit (MMN): Formation of an early (pre-attentive) auditory memory trace; automatic comparison process. MMN tests the integrity of the primary auditory memory network.	Deficit is present in the majority of SZ patients (not ameliorated by atypical antipsychotics). Presence of MMN deficit in first-episode patients uncertain.	$d \sim 1.0$ (meta-analysis) $d = 0.74$ (WAFSS)	Test-retest: $r = 0.78$ (duration MMN) $r = 0.53$ (frequency MMN) Intraclass $r = 0.9$ over 1 year.	No formal h^2 estimates available. Abnormality is present in a proportion of unaffected family members.	MMN is reduced in the 22q11 deletion syndrome. Possible association with rs4680 (COMT).
<i>Working memory updating/stimulus salience evaluation</i> Auditory P300 event-related potential Composite of: P3a (frontal); P3b (parietal)	Amplitude decrement and increased latency in SZ patients: one of the most consistent findings in the disorder.	Meta-analysis: $d = 0.89$ (amplitude) $d = 0.59$ (latency) WAFSS: $d = 0.91$ (amplitude)	Test-retest: $r = 0.81-0.91$ (2 weeks) $r = 0.59-0.61$ (1-2 years)	Twin studies: $h^2 = 0.60$ Unaffected family members similar to probands. Familial deficit most evident for P3a.	Amplitude decrement correlated with smaller left superior temporal gyrus. Genetic linkage to 6p24. Possible association with DISC1 and DRD2.
<i>Saccadic dysfunction</i> Antisaccade task (AS) Inhibition of reflexive prosaccade; performance of antisaccade to a mirror location	SZ patients: increased error rate; longer latencies to correct AS; reduced spatial accuracy	$d = 0.99$ (patients vs controls, WAFSS) $d = 0.99$ (relatives vs controls)	Test-retest: $r = 0.87$ (2 years) COGS inter-site study: $r = 0.77-0.96$ Deficit stable across clinical states	Twin studies: $h^2 = 0.57$ Error rate in unaffected family members: inconsistent data	Sensorimotor reprogramming; DLPFC, lateral interparietal area, supplementary eye field neurons. Linkage to 22q11 1-12 (COMT effect?)
c) Neuroimaging 3-D computational cortical surface mapping Reduced hippocampal volumes	Hippocampal volumes: Probands < unaffected co-twins < healthy subjects. Decrease in hippocampal size associated with cognitive deficit (memory dysfunction)	Not available	Not available	Twin studies: $h^2 = 0.42$	Hippocampal volume is environment- and activity-dependent. Possible role of DISC1, brain-derived neuro-trophic factor (BDNF) and translin-associated factor X (TRAX) genes.

Table III (Continued)

Domain/Tests	Association with disorder	Effect size	Trait stability/State independence	Heritability/ Segregation within Families	Underlying neurobiology /genetics
<i>Corpus callosum morphology</i>	Vertical (upward) displacement	Not available	Not available	Present in both affected and unaffected family members. Putative neuro-anatomical marker of biological vulnerability for SZ.	Genetic/neurodevelopmental origin likely.
<i>fMRI response to working memory tasks</i>	Exaggerated activation in right DLPFC in unaffected family members. Inefficient WM processing, similar to deficit in SZ patients.	Not available	Not available		Possible role of rs4680 (COMT).

Abbreviations. SZ, schizophrenia; C, controls; DLPFC, dorsolateral prefrontal cortex; WAFSS, Western Australian family study of schizophrenia.

ability and development of the brain throughout life (Purves et al. 2004). The theory was stimulated by accumulating and diversified evidence of an association between schizophrenia and events and conditions that can negatively influence early neurodevelopment, plus direct evidence of such abnormal development in schizophrenia patients (McNeil et al. 2000). Two important fields of evidence are neuromotor markers (NMMs) and minor physical anomalies (MPAs). NMMs (poor coordination and balance, involuntary movements, and deviant muscle power and tone), together with the Sensory, Reflex and Cognitive “neurological domains”, represent effective and inexpensive measures of abnormal functioning of the brain. Many different NMMs (both “hard signs” localized in the brain and non-localized “soft signs”) are consistently found in patients with schizophrenia versus normal controls. Sensitivity for schizophrenia is high: e.g., a degree of abnormality found in only 1% of normal controls characterised fully 80% of schizophrenia patients (McNeil and Cantor-Graae 2000). NMMs associated with adult schizophrenia appear as early as birth, and NMMs before 2 years of age seem to predict serious adult psychopathology in genetic high-risk groups. NMMs have a quantitative ‘trait’ component that remains significantly (if moderately) stable in individuals from middle childhood to adulthood, but that changes specific form over the life span. NMMs also have a “state” component related to current severity of psychosis, but are not primarily effects of neuroleptic treatment. Specificity for schizophrenia is low: NMMs are significantly increased, but to a lesser degree, in the mentally well relatives of schizophrenia patients and in patients with affective disorders, as well as many other mental and physical disorders. MPAs are minor structural changes of various bodily structures in the mouth, eye, ear, global head, hand and foot regions (e.g., epicanthus, steeped palate, adherent ear lobes, abnormal hair whorls, curved fifth finger, wide-spread toes). MPAs represent lasting signs of foetal maldevelopment occurring during the period of initial brain developmental (early pregnancy) and originating in the same structure (ectoderm) as the brain. Consistently increased rates of MPAs have been found in schizophrenia patients in many populations, with a substantial effect size across studies (Weinberg et al. 2006). The rate of MPAs in schizophrenia patients is unrelated to age, up to 60 years. Sensitivity for schizophrenia is notable, multivariate models of selected MPAs effectively discriminating ~80% of schizophrenia patients from ~80% of normal controls (McNeil et al. 2000). Specificity for schizophrenia is low, increased MPAs being found in healthy relatives as well

as patients with other neurodevelopmental disorders. Although significant increases of MPAs in schizophrenia are found in all body regions studied, major focus has concerned MPAs and morphometric shape anomalies in the craniofacial region. This region develops intimately with the brain, and researchers assumed that craniofacial abnormality parallels brain structural abnormality, a premise tested only recently. Unexpectedly, the craniofacial anomalies so clearly characterizing schizophrenia patients (vs. controls) seem to bear no systematic relation to patients' structural brain characteristics (temporal lobe and ventricular volumes, cortical thickness) in adulthood. Craniofacial deviations may possibly remain abnormal since early development, while brain structure responds to many co-temporaneous influences, continuously changing over time (Purves et al. 2004). Both genetic and environmental factors determine NMMs and MPAs, which are significantly increased in patients' biological relatives, and highly correlated within monozygotic twin pairs discordant for schizophrenia, yet MPAs in twins, and NMMs in patients' relatives are significantly related to obstetric complications. NMMs and MPAs represent effective, low-cost evidence of wide-spread, seemingly non-specific forms of early physical maldevelopment in schizophrenia and many other psychiatric and somatic disorders. Such maldevelopment seems to characterize a major proportion of schizophrenia patients (high sensitivity), this being useful for understanding the aetiology of the disease and possibly for individual treatment choice. However, NMMs and MPAs (as isolated risk characteristics) may be of limited value for identifying schizophrenia-prone individuals in the general population. Risk characteristics with low specificity for schizophrenia would yield high rates of falsely positive identification when screening for low prevalence disorders.

Functional brain systems

Cognitive vulnerability markers, neurocognition and fMRI

Generalized cognitive impairment, with varying degrees of deficit in all domains is thought to be a key feature in the schizophrenic psychoses, including global and selective verbal memory, nonverbal memory, bilateral and unilateral motor performance, visual and auditory attention, general intelligence, spatial ability, executive function, language, and interhemispheric tactile transfer (Heinrichs and Zakzanis 1998). However, highly predictive cognitive vulnerability markers that discriminate ill, stabilized or recovered patients from healthy individuals are still

lacking; not to mention the unaffected individuals at high risk at levels where they are intermediate between healthy subjects and patients (Saoud et al. 2000) despite neurocognitive, neurophysiological and neuroanatomic abnormalities. Two research paradigms that have been widely used to investigate vulnerability to psychosis are the genetic (i.e. offspring, non-psychotic co-twins or non-psychotic relatives siblings) and the clinical high risk strategy (i.e. personalities with attenuated psychotic like traits). Recently, the first quantitative reviews of cognitive impairments in the relatives of schizophrenia patients have been published (Sitskoorn et al. 2004; Szöke et al. 2005; Snitz et al. 2006; Gur et al. 2007). Snitz et al. (2006) found the largest effect sizes in the Trail Making Test (TMT) and performance measures from both simple and complex versions of the continuous performance task (CPT), whereas Szöke et al. (2005) found relatives of schizophrenic patients performing less well than controls on all executive tests analyzed (Stroop, Wisconsin Card Sorting Test and TMT) with larger effects for verbal fluency tests. Verbal memory recall and executive functioning showed larger effect sizes than attentional functioning (Sitskoorn et al. 2004). The Consortium on the Genetics of Schizophrenia (COGS) project (Horan et al. 2008) found working memory (WM) impairment pronounced in patients with schizophrenia, whereas the probands' relatives performed more poorly than comparison subjects only for the maintenance plus complex manipulation WM task. Although several cognitive tasks and variables are most impaired in patients with schizophrenia, they show only low specificity and sensitivity, and are not predictive for those who later develop manifest psychoses. This pointed to subgroups in schizophrenia with more distinct cognitive disturbances. The use of a high-risk strategy, which focuses on individuals considered to be at increased risk for psychosis (based primarily on the presence of brief limited and intermittent psychotic symptoms or recent decline in functioning), found these individuals performing intermediate to healthy controls and first episode psychosis patients, particularly on measures of verbal learning/memory, attention, executive abilities and general intellectual functioning. Only measures of verbal memory, spatial working memory and olfactory identification have demonstrated utility in predicting the transition to psychosis. By using neurocognitive vulnerability markers of transition in subjects at risk for psychosis, it might be possible to identify specific pathological processes that are active during the early course of schizophrenia. For this, brain imaging constitutes a powerful tool to explore the neurophysiological basis of the vulnerability to psychosis. Using functional MRI, one can study abnormal regional brain activa-

tion in high risk subjects during performance of specific cognitive tasks. Strikingly, and contrary to cognitive studies in high risk subjects, fMRI data have been reported essentially in adult relatives and only few studies have investigated child and adolescent relatives. In these fMRI studies, vulnerability to psychosis was associated with medium to large effect sizes when prefrontal activation was contrasted with that in controls (Fusar-Poli et al. 2007). Relatives or the co-twins of patients with schizophrenia and subjects with an "at risk mental state" appear to share similar neurocognitive abnormalities, but less severe than those observed in the first episode of illness. These abnormalities have mainly been described in the prefrontal and anterior cingulate cortex, the basal ganglia, hippocampus and cerebellum (Brans et al. 2008; Lawrie et al. 2008; Prasad and Keshavan 2008).

Post-mortem gene and protein expression

Functional genomics, by measuring changes in the expression of large numbers of genes and their products, enables data-driven hypotheses for the associations between functional gene groups and disease processes. In schizophrenia, microarray analyses of gene expression in post-mortem specimens, mostly the prefrontal cortex, have led to an assortment of diverse and concordant data. Concordant data sets have identified impairments in some major systems crucial for intact neuronal transmission and intercellular synaptic communication. The most consistent reduction and down-regulation of multiple genes encoding presynaptic proteins in the prefrontal cortex of schizophrenic patients were observed in transcripts such as *N*-ethylmaleimide sensitive factor (NSF), synaptosomal-associated protein (SNAP), synaptogyrin-1, synaptobrevin and clathrin (Mirnics et al. 2000; Knable et al. 2002; Vawter et al. 2002). Of special interest are the mitochondria, which are partly independent organelles with own DNA and a highly conserved oxidative phosphorylation system (OXPHOS). Significant protein profile alterations were identified in systems tightly linked to the mitochondrial energy production system, the malate shuttle system, the transcarboxylic acid cycle, and the aspartate and alanine metabolic pathway (Middleton et al. 2002), and in proteins of OXPHOS (Mehler-Wex et al. 2006). In a parallel transcriptomics, proteomics and metabolomics approach, genes related to energy metabolism and oxidative stress differentiated almost 90% of cases from controls in the prefrontal cortex (Bahn et al. 2001) with differential expression in mitochondria-related genes in the hippocampus (Altar et al. 2005). Data from multiple microarray studies report consistent down-regulation of oligo-

dendrocyte and myelin related transcripts including myelin-associated glycoprotein, proteolipid protein 1 (PLP1), myelin-associated oligodendrocyte basic protein, myelin oligodendrocyte glycoprotein, myelin basic protein, and 2',3'-cyclic nucleotide 3'-phosphodiesterase (Katsel et al. 2005; Hakak et al. 2001; Sugai et al. 2004; Tkachev et al. 2003). This supports recent suggestions of white matter changes in schizophrenia associated with altered structure and density of oligodendrocyte and dysfunction in myelination (Katsel et al. 2005; Davis et al. 2003). Finally, alterations in multiple other genes related to additional systems and pathways have been discovered, among them transcripts related to ubiquitination of proteins, the GABA and glutamate related pathways, G protein signalling, the wnt-GSK-3 pathways as well as to the immune/chaperone system. Although high-throughput microarray profiling in brain tissue enables the generation of novel hypothesis or corroborate current hypotheses related to the pathomechanisms of schizophrenia, gene expression does not consistently correlate with protein expression or function, due to post-transcriptional and post-translational modifications as well as interactions of gene products with other proteins or small molecules. Therefore, it remains to be demonstrated in each case, if the deviant expression of functionally related genes correlates with disease pathogenesis and pathologies. Combining proteomic data (mass spectroscopy and expression scanning) with microarray gene expression will tremendously contribute to uncover disease related groups of genes. Using two-dimensional gel electrophoresis Edgar and colleagues (2000) identified 108 differentially expressed proteins in the hippocampal proteome of schizophrenic patients, including diazepam binding inhibitor (DBI), mitochondrial manganese superoxide dismutase (MnSOD), collapsing response mediator protein-2 (CRMP-2), which regulates axonal growth and polarity, and t-complex protein 1 (TCP-1) acting as a chaperone protein. In the frontal cortex almost half of the altered proteins identified by proteomics were associated with mitochondrial function and oxidative stress responses thus mirroring the transcriptional data (Bahn et al. 2001). Only a small number of risk genes generated by linkage and association studies were identified by microarray studies, and thus, it remains unclear whether these findings are epiphenomena related to disease and treatment or true biological markers. Most microarray studies have been performed in post-mortem brain specimens, but in order to turn these genes into biomarker it remains to be demonstrated that their expression is also altered in a more accessible tissue, correlates with disease course and symptoms, and shows disease specificity. Expression of genes, protein levels

and function are tissue and environment dependent, adding to the complexity of the identification of a peripheral biomarker. Indeed, several microarray studies in blood cells have identified genes that discriminate between schizophrenia patients and controls but did not always parallel putative CNS changes in schizophrenic psychoses (Gladkevich et al. 2004; Tsuang et al. 2005; Vawter et al. 2004; Glatt et al. 2005).

Cerebrospinal fluid

Cerebrospinal fluid (CSF) proteomics and lipidomics play important roles in the discovery of potential biomarkers of schizophrenia. Metabolic stress, increased oxidation and changes in macromolecular composition of the CSF of patients with schizophrenia compared with normal subjects are some biochemical processes that suggest the beginning or progression of the disease. Biochemical changes in CSF of cases are linked to changes in the expression, function or modifications of several proteins. These include proteins involved in oxidation, inflammation, immune response, receptor function, neurotransmitter biosynthesis or organization of brain membranes. Analyses of CSF proteins (Huhmer et al. 2006) are proposed to better define the pathophysiology of schizophrenia. Lipids are important in energy metabolism, neurotransmission, receptor function and cellular signalling. A promising role for lipids in schizophrenia is emerging from dietary and epidemiological studies showing favourable outcomes in omega-3 fatty acid supplemented diets, and several enzymes were found important in the transport, biosynthesis and oxidative metabolism of lipids in CSF (Fonteh et al. 2006; Table IV). Targeted and untargeted lipidomics studies are aiding advances in understanding of biochemical pathways in schizophrenia and other brain diseases (Adibhatla et al. 2006). In a proteomic approach to the CSF samples, potentially specific changes were observed in a peptide that mapped to the VGF protein and in several peptides that mapped to transthyretin (Huang et al. 2006). Interestingly, both VGF (nerve growth factor-inducible neuropeptide) and transthyretin, demonstrated parallel alterations in postmortem brain tissue of patients with schizophrenia. Several CSF proteins and lipids pathways are important in brain physiology (Wenk 2005) and have the potential of being useful biomarkers of schizophrenia (Law et al. 2006; Table IV). While changes are reported for these molecules, usually, only small sample sizes were studied, pointing to the need of more rigorous clinical studies. The post-genomic era has seen improvements in compositional analyses of proteins and lipids, largely driven by mass spectrometry technology and bioin-

formatics. For CSF analyses, detection of low-abundance proteins normally involves extensive sample preparation and enrichment strategies followed by multidimensional chromatography and then high-resolution mass spectrometry (Huhmer et al. 2006), that enables detection of thousands of CSF proteins, including dopamine-, serotonin-, acetylcholine- and glutamate-pathway receptors, and metabolic enzymes. Compositional studies have identified some CSF proteins that change in CSF of schizophrenic patients (Harrington et al., 2006). There is still a need for validation of putative protein biomarkers. Validation of candidate biomarkers requires rigorous quantification by either isotope dilution mass spectrometry or antibody-based screening methods. Studies of biochemical pathways are likely to reveal changes in levels or post-translational modification of proteins in CSF from schizophrenics compared with normal subjects. Since mass spectrometry is expensive and needs trained personnel, its contribution may be limited to discovery and validation while high throughput methods using chip technology are likely to find applications in clinical diagnosis. Compositional or targeted study of CSF proteins, especially those associated with neurotransmitter function and the generation of lipid signalling molecules may provide early clues on the pathology of schizophrenia.

Chromosomal loci and genetic markers

Despite considerable efforts using linkage and association studies, the genetic basis even in familial forms of schizophrenia remains elusive, and specific molecular markers to discriminate persons-at-risk or at least those with the clinical presentation of schizophrenia are still lacking. Even in genome-wide linkage approaches with multiple multigenerational families, scan by scan brought disappointing evidence that the genetic complexity of the disease is tremendous, if dealt as a single disorder and if not rigorously subdivided in distinct phenotypes. In addition, potential gene-gene and gene-environment interactions made the identification of susceptibility genes even more difficult. In the era of genome-wide association studies and large-scale genotyping technologies, one-polymorphism genetic association studies are no longer state of the art and were, thus, not included in the Task Force paper. A systematic meta-analysis of genetic association studies and database of polymorphisms proposed to be associated with schizophrenia was created recently (Allen et al. 2008; <http://www.schizophreniaforum.org/>).

In two meta-analyses on whole-genome linkage scans, the results were only partially overlapping due to methodological differences: Badner and

Table IV. CSF proteins and lipids involved in the pathophysiology of schizophrenia. Several CSF proteins and lipids are important in transport, nerve cell excitability, nerve cell growth and neuron secretion. These functions of lipids may have significant physiologic ramifications in schizophrenia (pers. comm. A. Fonteh)

Proteins	Lipid substrates and metabolites	Functions or putative mechanisms of action
apolipoproteins, apolipoprotein receptors, ATP-cassette binding proteins, LCAT, CETP δ -6 or F-5-desaturases, elongase	Phospholipids, cholesterol, fatty acids Polyunsaturated fatty acids (¹ PUFA)	Uptake and transport of lipids, nerve cell growth/regeneration Synthesis of ¹ PUFAs, membrane fluidity, receptor function
Cyclooxygenases, lipoxygenases, monooxygenases, prostaglandin synthases Phosphoinositide kinases	Polyunsaturated fatty acids (AA, EPA, DHA), prostaglandins, leukotrienes Phosphotidylinositols, phosphoinositides	Signal transduction, lipid mediators of inflammation and pain, neurotransmission Signalling molecules, receptor function, vesicle formation, fusion, trafficking exocytosis, neurotransmitter secretion
Transferases, transacylases, phospholipases (PLA ₂ , PLC, PLD), platelet-activating factor-acetyl hydrolases (PAFAH), sphingomyelinase Fatty acid amide hydrolase (FAAH), monoglyceride lipases	Phospholipids, lyso-phospholipids, sphin-gosine-1-phosphate, diacylglycerols, phos-phatidic acid, inositol phosphates, endocan-nabinoids, platelet-activating factor Fatty acid amides (e.g., arachidonoyl etha-nolamide, AEA), and fatty acyl glycerols (e.g., arachidonoyl-glycerol) known as en-docannabinoids	Physical properties of cell membranes, endocytosis, vesicle formation, secretion, plasticity, precursors of lipid mediators of inflammation and pain, signalling molecules Signalling molecules, ligands of G-protein-coupled receptors (CB1 and CB2) receptor. Implicated in pain, depression, cognition, sleep, synap-tic plasticity, feeding and behavioural effects

Abbreviations: LCAT, lecithin-cholesterol acyl transferase; CETP, cholesterol ester transfer protein; PUFA, polyunsaturated fatty acids; AA, arachidonic acid; EPA, eicisapentaenoic acid; DHA, docosahexaenoic acid; CB, cannabinoid.

Gershon (2004) referred to published linkage data combining the reported P values of individual studies, after correcting each value for the size of the region containing a minimum P value, whereas Lewis and colleagues (2005) used a rank-based genome scan meta-analysis method with reanalysis of initial genotyping data sets of the respective scans. The first found strongest evidence for schizophrenia on chromosomes 8p, 13q and 22q, whereas the second meta-analysis found the best evidence for linkage on chromosome 2q and with less significance on chromosomes 1q, 3p, 5q, 6p, 8p, 11q, 14p, 19, 20q and 22q (Figure 4). However, within the chromosome 2q candidate region no risk genes were established.

Complementary to the chromosomal loci derived in the meta-analyses, several other confirmed loci with appropriated candidate genes appeared (Table V). Analysis of a balanced translocation (1;11)(q42.1; q14.3) cosegregating with schizophrenia in a large Scottish family lead to the identification of the gene disrupted-in-schizophrenia 1 (DISC1), which is supposed to play a role on cytoskeletal regulation, brain development and neurocognition (Blackwood et al. 2001). However, haplotype studies remained ambiguous regarding association with disease. The

G-protein signalling 4 (RGS4) gene, located on chromosome 1q21–q22, regulates G-protein-mediated signalling via neurotransmitters such as dopamine or glutamate, and showed altered expression in the prefrontal cortex of patients with schizophrenia (Chowdari et al. 2002), but again the genetic association was only replicable in a fraction of studies. Analysis of the candidate chromosomal region 6p24–22 revealed dysbindin (DTNBP1, Straub et al. 2002). An association with a clinical phenotype defined by negative symptoms and with general cognitive ability was reported, but results were challenged by the fact that DTNBP1 codes for type 7 of the rare autosomal recessively inherited Hermansky-Pudlak syndrome (HPS; oculocutaneous albinism and abnormal lysosomal ceroid storage without evidence for psychosis). Neuregulin 1 (NRG1) on 8p22–p11 belongs to a gene family with a wide range of functions in the central nervous system as neurodevelopment and expression of neurotransmitter receptors (Stefansson et al. 2002). Again replication in haplotype studies was equivocal, and the pathophysiological mechanism is still unclear. *G72/G30* on chromosome 13q14–q32 has been repeatedly associated with schizophrenia and with weaker evidence with bipolar disorder (Chumakov et al. 2002). *G30*

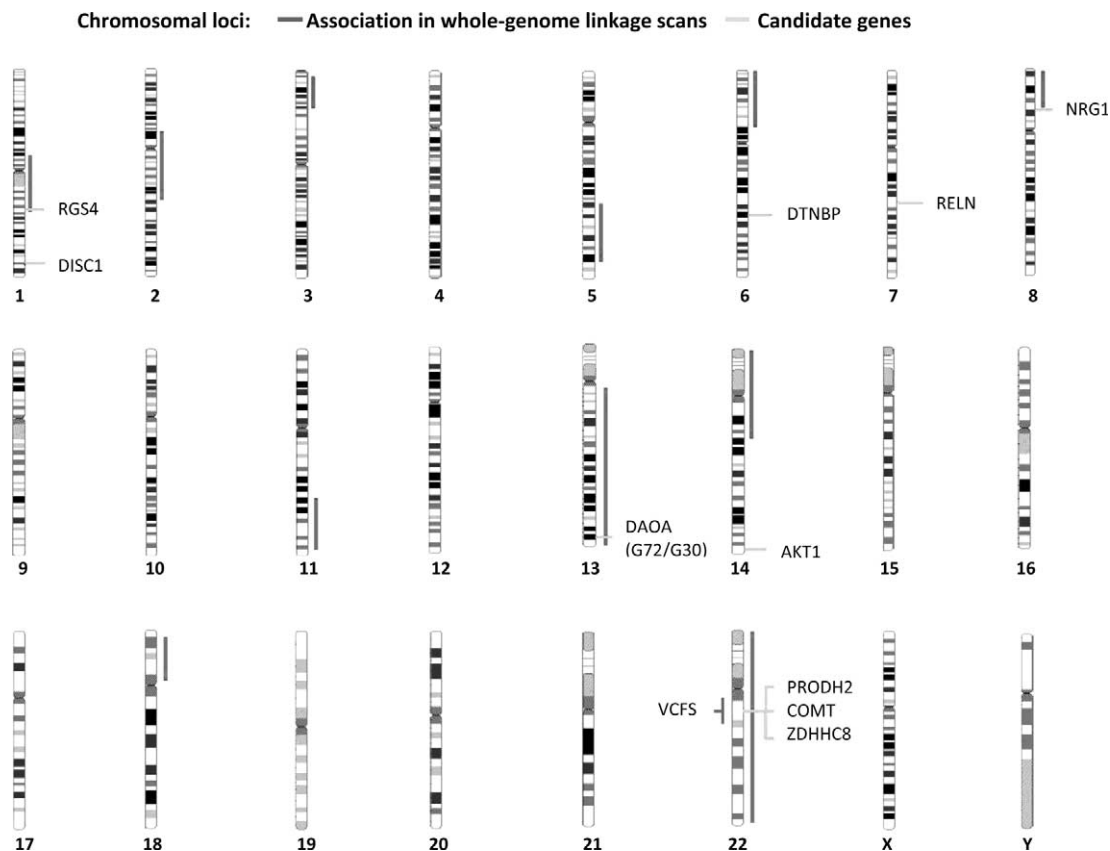


Figure 4. Chromosomal susceptibility loci for schizophrenia derived by meta-analyses of genomewide linkage scans, and proposed risk genes (pers. comm. M. Gawlik).

Table V. Candidate genes for schizophrenic psychoses and strength of the biological evidence

gene	chromosomal localisation	Weighting the evidence (0–5+)* for			
		genetic association with schizophrenia	linkage to gene locus	biological function	altered expression in schizophrenia
RGS4	1q23.3	+++	+++	synaptic signalling	yes
DISC1	1q42.2	++++	++	neuronal migrating	not known
DTNBP1 (Dysbindin)	6p22.3	+++++	++++	glutamate release	yes
NRG1(Neuregulin)	8p12	+++++	++++	neuronal migration, glutamate signalling	yes
d-amino-acid oxidase (DAO)	12q24	+++	++	glutamate signalling	not known
d-amino-acid oxidase activator (G72/G30)	13q33.2	+++	++	glutamate signalling	not known
AKT1	14q32.33	+	+	synaptic signalling	yes
COMT	22q11	++++	++++	dopamine metabolism	yes

*according to Harrison and Weinberger (2005); chromosomal localisation acc. to UCSC database (genome.ucsc.edu/)

Note: effect size does not exceed “minor gene” effects (estimated OR 1.5–1.8).

and *G72* overlap on complementary chromosomal strands with opposite orientations. A regulator function was suggested for *G30*, whereas *G72* was initially placed in the glutamate pathway, but turned out to be a modulating factor of mitochondrial function. *AKT1* on chromosome 14q32 locates in a region with weak evidence for linkage, and is mainly supported by pathophysiological considerations (Emamian et al. 2004). *AKT1* is thought to operate as a protein kinase influencing several signal transduction pathways in the central nervous system. The reduced expression of *AKT1* in post mortem brain specimen of patients with schizophrenia is thought to alter neurotransmission and neurogenesis. Again, haplotype studies missed consistent replication. Chromosome abnormalities associated with schizophreniform psychoses were reported for the velocardiofacial syndrome (VCFS) with a microdeletion at 22q11. Since the psychopathology of carriers of the microdeletion is still under debate (Pulver et al. 1994; Verhoeven et al. 2007), a systematic screening for the deletion has never been implemented in psychiatric diagnostic procedure. The microdeletion region on chromosome 22q11 contains the genes for catechol-*O*-methyltransferase (*COMT*), proline dehydrogenase (*PRODH2*), and the brain expressed putative palmitoyltransferase *ZDHHC8*, implicated in schizophrenia pathogenesis. Most of the association studies at the 22q11 deletion region have been performed on the *COMT* gene involved in DA catabolism, and within *COMT* on the functional polymorphism Val158/108Met (rs4680). Throughout the meta-analyses carried out, there was only a weak association of the variant to schizophrenia (Sand et al. 2006).

During recent years, numerous candidate genes have been proposed as risk factors for schizophrenia (Allen et al. 2008), *AKT1*, *APOE*, *BDNF*, *CHRNA7*, *COMT*, *DRD2*, *DRD3*, *DTNBP1*, *G72*, *NRG1*, *RELN*, *RGS4*, often related to the patho-

physiological hypotheses on schizophrenia the respective research group is pending (see Figure 2). The biology of these genes are expected to affect glutamate and GABA signalling in the cortex, to converge with dopamine on the cellular physiology of microcircuit behaviour, and to affect both cortical synapses and network stability. However, it is still to be determined whether these minor genes act in a simple additive model or in a complex polygenic inheritance network towards instability and increased risk for manifesting schizophrenia.

Assuming schizophrenia as a complex genetic disease, the debate over common versus rare variants in psychiatric genetics is renewed in the era of genome-wide association studies. The first hypothesis proposes that common-disease liability results from multiple genetic variants of small effect but relatively high frequency (common disease-common variant hypothesis). The opposed hypothesis is that rare de novo variants (sporadic cases) or rare highly penetrant variants in families, which strongly segregate schizophrenia (familial cases), are causative factors. However, the failure of linkage studies in schizophrenia to detect causative point mutations stands vis-à-vis to the failure of the genetic association approach to produce consistent and reproducible results in candidate genes. Modern biomedical technology (Illumina® or Affymetrix® Gene Chip platforms) allow dense genome-wide array based studies to proof both the common vs. the rare variant hypothesis.

Among the six genome-wide association studies (GWAS) on schizophrenia – published up to now – three studies have been based on DNA pooling (Mah et al. 2006; Kirov et al. 2009; Shifman et al. 2009), where in a single quantitative assay the estimated allele frequencies of pooled DNA from cases are compared to those of controls. The most interesting finding was a female-specific association

between *reelin* (*RELN*) and schizophrenia with $p = 8.8 \times 10^{-7}$ in a meta-analysis of different study samples (Shifman et al. 2009). In the first study based on individual genotyping (178 cases and 144 controls), the strongest results ($p = 3.7 \times 10^{-7}$) were derived at chromosome 3q25 within the vicinity of two genes, *colony stimulating factor 2 receptor alpha* (*CSF2RA*) and *short stature homeobox isoform b* (*SHOX*) (Lencz et al., 2007). The second GWA with individual genotypes was based on an ethnically heterogeneous sample and achieved no results of genome-wide significance (Sullivan et al. 2008). The largest study so far, using the Affymetrix GeneChip 500K Mapping Array (O'Donovan et al. 2008), genotyped a UK-sample of 479 cases with DSM-IV schizophrenia in comparison to 2,937 subjects from the Wellcome Trust Case Control sample, and followed up 12 putative loci in international replication sets of approximately 15,000 cases and controls. In these cohorts and a combined bipolar and schizophrenia UK-sample, six single nucleotide polymorphisms (SNPs) supported association, with the strongest evidence for SNP-marker rs1344706 at the zinc finger *ZNF804A* locus on chromosome 2q32.1 ($p = 1.61 \times 10^{-7}$). The encoded protein is reported to bind N-terminal sequences of ataxin-1, but its function is fairly unknown (Lim et al. 2006). Two further SNP-markers are located intergenic (SNP rs1602565, rs7192086), whereas the remaining three markers (rs6490121, rs9922369 and rs3016384) are situated intronic within *NOS1*, *RPGRIP1L*, and *OPCML*. Odds-ratios varied between 1.03 (rs6490121 at the *NOS1* locus) and 1.20 (rs7192086 at *RPGRIP1L*) in the analysis of the combined schizophrenia and bipolar samples.

Genome-wide structural variation, i.e. small duplications or deletions, copy number variants (CNVs), or inversions were assessed in four recently published studies (International Schizophrenia Consortium 2008; Stefansson et al. 2008; Walsh et al. 2008; Xu et al. 2008). Walsh and colleagues (2008) found the frequency of CNVs to be 15% in 150 probands with schizophrenia compared to 5% in 268 controls, a follow-up analysis of 83 childhood-onset cases provided modest evidence of association for a set of rare, largely inherited CNVs. The group of Karayiorgou (Xu et al. 2008) examined de novo CNVs in a set of 359 trios, and in a subset of sporadic cases, they observed an eight-fold increase (10 vs. 1.5%) of de novo CNVs over controls, whereas those with a positive family history failed to show de novo CNVs. Stefansson and colleagues (2008) identified 66 de novo CNVs in 2160 trios, three deletions at 1q21.1, 15q11.2 and 15q13.3 generated evidence for association. At last, the International Schizophrenia Consortium (2008) reported on a modest, 1.15-

fold increase in overall CNV frequency in 3,391 cases and 3,181 controls. Corresponding to the findings of Stefansson and colleagues, they found significant association with deletions on 15q13.3 and 1q21.1. Given the heterogeneity of disease, however, drawbacks with these approaches cannot be excluded. Further studies will reveal the relevance and importance of these recent findings (Maher et al. 2008).

Currently accessible analytical systems

Immune cytokines

In a sub-proportion of patients with treatment resistance and clinical deterioration the decline seems associated with persistent, recurrent sensitization of the phasic and tonic dopamine system, and with serotonin receptor dysfunction and NMDA receptor hypofunction or oxidative stress leading to glutamate induced neurotoxicity. In this scenario, cytokines are multifunctional proteins that are released by a variety of cells both in the brain (neurons and glia) and elsewhere in the body (macrophages and lymphocytes; Figure 5). Cytokines play a crucial role in cell-to-cell communication in the immune system and in the interaction between the immune system and the CNS. The activities of cytokines are mediated through specific receptors expressed on the cell surface or through soluble receptors shed from cells. Cytokines modulate the activity, differentiation, and survival of neuronal cells in the neurodevelopmental stage of brain. Cytokine over-expression in the brain is an important factor in neurotoxicity and neurodegeneration. Cytokines interact with neuroendocrine systems, regulate synaptic proteins and structural molecules in the brain of affected individuals. Specific cytokine polymorphisms differentially modulate cytokine-mediated neuronal injury, and may represent susceptibility genes for development of schizophrenia following viral infection or brain injury during neurodevelopment (Marx et al. 2001). The TNF- α gene, located on chromosome 6p, exhibits several polymorphism associated with schizophrenia, certain alleles of IL-1b, IL-1a, and IL-1RA are significantly increased among cases compared to controls (Katila et al. 1999). There is some evidence that a functional link exists with IL-2, since IL-2 levels are elevated in CSF and plasma of neuroleptic-free patients, increased CSF levels of IL-2 predict the expression of psychotic symptoms and plasma levels of IL-2 as well as the dopaminergic metabolite homovanillic acid are increased coincidentally with occurrence of positive symptoms, and significantly decreased following treatment with haloperidol (Kim et al. 2000). Conversely, IL-6 levels are associated with both negative symptoms

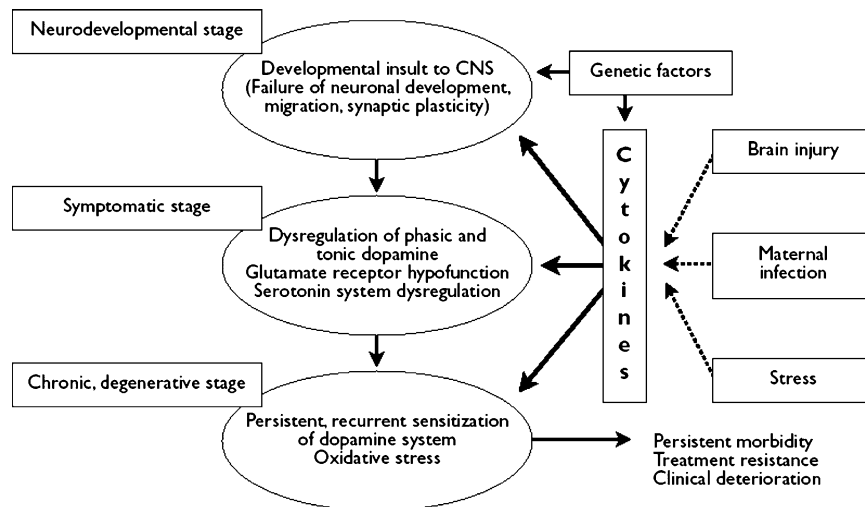


Figure 5. The potential role of cytokines in the pathophysiology of schizophrenia (pers. comm. YK Kim).

and duration of illness, and elevated plasma IL-6 in schizophrenia correlate to the development of cerebral atrophy. Cytokines influence central monoamine activity in a cytokine-specific manner (Zalcman et al. 1998). Hypothalamic and hippocampal norepinephrine utilization and dopamine turnover in the prefrontal cortex is modified by IL-2, while IL-6 induces profound elevation of serotonin and mesocortical dopamine activity in the hippocampus and prefrontal cortex. IL-1, in contrast, induces a wide range of central monoamine alterations.

Cytokines, such as IL-1b, IL-2 and IFN-g, reduce the production of serotonin by stimulating the activity of indoleamine-2,3-dioxygenase (IDO), an enzyme that converts tryptophan, the precursor of 5-HT, to kynurenine (Guillemin et al. 2001; Sakash et al. 2002). The kynurenine is again metabolized (Dang et al. 2000) into quinolinic acid and kynurenic acid. The cytokine-serotonin interaction that leads to a challenge between neurodegenerative quinolinate and neuroprotective kynurenate in the brain may also explain the neurodegeneration in schizophrenia. In agreement with these ideas, recent studies have shown elevated levels of kynurenic acid in the CSF (Erhardt et al. 2001) and in the cortical brain tissue of schizophrenia patients (Schwarcz et al. 2001). These findings suggest that the elevated levels of kynurenic acid in schizophrenia may induce hyperactivity of the mesocorticolimbic dopamine system (Erhardt and Engberg 2002). Therefore, the involvement of the interactions between cytokines, the tryptophan degradation pathway, and neurodegeneration in schizophrenia needs further exploration.

Lymphocytes and platelets

Studies over the past few years have yielded persuasive evidence that T cells recognizing abundant brain

antigens are needed for maintaining brain plasticity (cognition and neurogenesis), for protection, and repair (Kipnis et al. 2004; Ziv et al. 2006). When this protective response gets out of control, it may result in adverse effects, such as exacerbation of the damage or development of an autoimmune disease (Hauben et al. 2001; Yoles et al. 2001). Although the overall autoimmunity in schizophrenic patients might be highly activated, the specific T-cell clones that are needed to maintain brain plasticity and homeostasis are lacking. In line with the hypothesis of immune aberrations, patients with schizophrenia show altered ratios of CD4⁺ T cells and CD8⁺ T cells, increased proliferation of activated lymphocytes (blast cells), heightened reactivity of T cells to mitogenic stimuli, and elevated expression of activation markers and receptors for neurotransmitters on blast cells (Cosentino et al. 1996; Rapaport et al. 1993). Patients with early onset and prominent negative symptoms might be etiologically associated with a deficiency of relevant autoimmunity, since early onset schizophrenia seems accompanied by prominent clinical decline reflected by progressive neuroradiological changes, indicative of neuronal loss (Bellino et al. 2004; Sato et al. 2004). This contrasts to the later onset and less prominent negative symptoms in female patients, which are thought to exhibit a more active autoimmune system than males (Lewine et al. 1981). In patients with schizophrenia a decrease in myelin basic protein (MBP)-specific autoantibody reactivity is proposed compared to population controls (Jankovic et al. 1991). In line with these findings, the response to MBP in medicated schizophrenic patients is on average significantly weaker than in controls. This contrasts with the observation that individuals with schizophrenia are more responsive than community controls to various immunological stimuli (Müller

et al. 1991). These findings seem to support the hypothesis that, whereas the overall immune response in patients is higher than that in the general population, autoimmune clones or at least those specific to MBP are either lost or kept in an anergic state. Thus, individuals affected with schizophrenia might undergo a selective early loss of autoimmune T cell clones specific to CNS myelin proteins. By addressing the role of mitochondria in peripheral systems, which are partly independent organelles with own DNA and an oxidative phosphorylation system (OXPHOS), specific alterations in gene and protein expression of three subunits of the first complex of the OXPHOS were detectable in blood cells, which was paralleled by impaired complex I enzymatic activity (Ben-Shachar 2002; Ben-Shachar et al. 1999; Mehler-Wex et al. 2006; Huang et al. 2006). In addition, complex I activity in blood cells showed disease course dependent alterations, suggesting complex I as a (potential) biomarker for schizophrenia. However, it is important to note that antipsychotic drugs can alter both the expression and function of genes and protein that are implicated in schizophrenia.

Endogenous intoxication

Endogenous intoxication (endotoxycosis) is a pathophysiological process that is characterized by the formation and accumulation in tissues and body fluids of different substances and metabolites in excessive concentrations or in forms that are not characteristic for the normal metabolism (Uzbekov et al. 2006). Increased levels of middle-mass endotoxic molecules (MMEM), monoamine oxidase (MAO) activity, lipid peroxidation (LPO), impairments in functional albumin, have contributed to the development of endotoxycosis. MMEM is a fraction of different blood plasma substances in the range of 300–5,000 Da. In patients' plasma MMEM concentration exceeded control level for 2–3 times, and platelet MAO-B activity (substrate benzylamine) was increased for 0.5–2 times, reflecting disturbances in monoaminergic systems. These changes refer to the damage of membrane structures and appearance of toxic end products (MMEM fraction) in blood as well as they may be indicative that disease is accompanied by increased production of H₂O₂ that promotes activation of LPO. Malondialdehyde concentration reflecting the activity of LPO was elevated in patients for about 50%. FES patient's serum SSAO activity was significantly decreased for about 30%. Products of SSAO activity, such as acrolein, methylglyoxal, etc., contribute in MMEM fraction. Base-line serum MMEM content can serve as a predictor of the efficacy of plasmapheresis treatment in schizophrenic patients resistant to

psychopharmacotherapy. The decrease of specific binding parameters of the probe (*N*-carboxyphenylimide of dimethylaminonaphthalic acid) that selectively binds in serum only to albumin in patients with schizophrenia points to the damage in main albumin functions (transport and detoxification) and aggravation of endotoxycosis. Fluorescent decay studies on S-60 synchrotron have revealed a redistribution in albumin between long-lived and short-lived molecules of the respective probe with increase of the latter (Gryzunov et al. 2000). Fluorescence quenching constant for the probe bound to albumin was $2,48 \pm 0,17$ l/mol in patients versus $4,65 \pm 0,37$ l/mol in volunteers (Uzbekov et al. 2008). In addition, there appears a decrease of albumin accessible SH-groups (Sokolova et al. 2005) indicating conformational changes of albumin molecules in schizophrenia. Future studies will have to demonstrate the components of MMEM in order to get further insights into the mechanisms of such endogenous intoxication.

Functional genomics

With advanced molecular techniques and by applying the modern methodologies of proteomics, new data have elucidated changes in peripheral systems on the mRNA and proteomics level which might be useful in defining biomarkers with diagnostic, therapeutic and prognostic relevance. Proposed alterations on the mRNA level include increased levels of D3 dopamine receptor mRNA (Ilani et al. 2001) and decreased levels of CHRNA7 mRNA (Perl et al. 2003) in peripheral blood lymphocytes as well as increased mRNA levels of the mitochondrial complex I 24-, 51- and 75-kDa subunits in whole blood cells as well as proteins of the former two subunits (Dror et al. 2002; Mehler-Wex et al. 2006). These alterations were not due to treatment with antipsychotic drugs or smoking, since drug-naïve and non-smoking patients were included in these studies as well and exhibited a similarly altered mRNA levels. Furthermore, advanced gene chip approaches allow hence for hypothesis-generating approaches, scanning hundreds and thousands of different possible mRNA markers at the same time in a high-throughput setup. This methodology will contribute to a faster identification of further possible mRNA markers in schizophrenia. cDNA microarrays have been only used in a proposed animal model of schizophrenia (MK-801) for RNA analysis. Genes whose expression was significantly up- or down-regulated included cytochrome C oxidase subunits, plasma membrane calcium-transporting ATPase (PMCA), several different kinases and synaptotagmin isoforms (Paulson et al. 2003). However, this study was based on the analysis of brain

samples, and not of peripheral systems. The same study also included a proteomic analysis which revealed altered levels of proteins such as different heat shock proteins, stathmin and enolases. Interestingly, a similar hypothesis-free approach has already been successfully used in clinical proteomics studies of patients suffering from schizophrenia. Using profiling techniques such as SELDI-ToF-MS and/or MALDI-ToF MS (surface enhanced laser desorption ionisation-time of flight-mass spectrometry, matrix assisted laser desorption ionization-time of flight-mass spectrometry, respectively), it is possible to screen peripheral systems such as blood serum for general protein patterns which differ between patient and control groups (Lakhan 2006). This approach has already been successfully used in cancer research and some researchers are beginning to apply this technology to psychiatric disorders. Analyses of CSF proteins in schizophrenic patients revealed significant alterations of the apolipoprotein A-IV content, while another study focussing on the plasma proteome of such patients uncovered increased levels of acute phase proteins (Hünnerkopf et al. 2007). In a recent study applying a so-called label-free quantitative proteomic approach to samples obtained from schizophrenic patients as well as healthy controls, hundreds of potentially altered serum proteins were identified, thus suggesting that this technology can be used to profile a high number of proteins across large sets of complex samples, such as those obtained from patients suffering from psychiatric disorders (Levin et al. 2007).

Conclusions

Due to the complexity of a mental disorder such as the schizophrenic psychoses, the identification of reliable peripheral markers has, so far, been elusive. It is important to note that the effect size of any biological change observed in schizophrenia will remain rather small, if the disease is treated as a single entity. This is partially due to the lack of established nosological systems, and partially due to the fact that mental disorders are brain disorders whose neuropathology might not be reflected in peripheral systems. It remains to be seen whether these findings regarding possible peripheral biomarkers for schizophrenic psychoses can be confirmed in future independent studies in large samples. If so, these urgently needed markers could contribute to a more reliable diagnosis of the schizophrenic psychoses and help to identify and differentiate different sub-phenotypes. Such biomarkers also would help to identify individuals with increased risk for this devastating mental disorder and contribute to early diagnosis and intervention. They furthermore could

contribute to more individualised treatment strategies with increased efficacy and reduced side effects and also possibly be used as prognostic predictors.

Databases

HUGO Gene Nomenclature Committee EMBL, Hinxton (HGNC), UK: www.genenames.org (approved gene names); UCSC Genome Bioinformatics group, University of California, Santa Cruz, USA: www.genome.ucsc.edu/ (chromosomal localisations, nucleotide positions); NCBI SNP database, National Center for Biotechnology Information, US National Library of Medicine, Bethesda, USA: www.ncbi.nlm.nih.gov/SNP/ (single nucleotide polymorphisms, SNP); International HapMap Project: www.hapmap.org/ (single nucleotide polymorphisms, SNP); UniProtKB/Swiss-Prot, Swiss Institute of Bioinformatics (SIB): www.expasy.org/ (Protein)

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