Consensus paper of the WFSBP Task Force on Biological Markers: Criteria for biomarkers and endophenotypes of schizophrenia part II: Cognition, neuroimaging and genetics

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ABSTRACT

Objectives: Schizophrenia is a group of severe psychiatric disorders with high heritability but only low odds ratios of risk genes. Despite progress in the identification of pathophysiological processes, valid biomarkers of the disease are still lacking.

Methods: This comprehensive review summarises recent efforts to identify genetic underpinnings, clinical and cognitive endophenotypes and symptom dimensions of schizophrenia and presents findings from neuroimaging studies with structural, functional and spectroscopy magnetic resonance imaging and positron emission tomography. The potential of findings to be biomarkers of schizophrenia is discussed.

Results: Recent findings have not resulted in clear biomarkers for schizophrenia. However, we identified several biomarkers that are potential candidates for future research. Among them, copy number variations and links between genetic polymorphisms derived from genome-wide analysis studies, clinical or cognitive phenotypes, multimodal neuroimaging findings including positron emission tomography and magnetic resonance imaging, and the application of multivariate pattern analyses are promising.

Conclusions: Future studies should address the effects of treatment and stage of the disease more precisely and apply combinations of biomarker candidates. Although biomarkers for schizophrenia await validation, knowledge on candidate genomic and neuroimaging biomarkers is growing rapidly and research on this topic has the potential to identify psychiatric endophenotypes and in the future increase insight on individual treatment response in schizophrenia.

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Introduction

The phenotype of schizophrenia is still based solely on clinical criteria, with continued changes proposed in each revision of the International Statistical Classification of Diseases and Related Health Problems (now in its 10th revision; WHO 2010) and the Diagnostic and Statistical Manual of Mental Disorders (now in its 5th revision; APA 2013). Symptoms vary considerably during the course of the disorder because of the heterogeneous composition of psychopathological symptoms, which include hallucinations, delusions, disturbances of self, thought disorders, psychomotor abnormalities, so-called “positive”, “negative” and affective symptoms and the widespread presence of cognitive deficits (Falkai 2011). Outcome also varies considerably: one episode and remission are seen in 10% of patients, several episodes and remission in 30%, several episodes with stable residuum in
10% and a chronic course with increasing residual symptoms in 40% (Watt et al. 1983; Marengo 1994; Lambert et al. 2010). Schizophrenia is considered to be either a syndrome or a group of different disorders, the "group of schizophrenias", as described by E. Bleuler himself (Beckmann 1999). Despite tremendous efforts during the last decade (Stöber et al. 2009), valid biomarkers for improved early diagnosis and prediction of outcome and treatment response are lacking or still being evaluated. The most promising progress has been made in the field of cognition and endophenotypes (Demtl and Habel 2011; Kalia and Costa 2015), neuroimaging (Atluri et al. 2013) and genetics (Vawter et al. 2011); this progress will probably lead to improved diagnosis and development of innovative and personalised treatment strategies (Ozomaro et al. 2013).

**Heritability of schizophrenia**

Family, twin and adoption studies (Cardno and Gottesman 2000; Sullivan et al. 2003; Wray and Gottesman 2012) and estimates from genome-wide association (GWA) studies (Gusev et al. 2014) demonstrate evidence for a strong genetic component in the aetiology of schizophrenia. The risk of developing the disease increases with the genetic relatedness to an individual with the disorder. Third-degree relatives have a risk of approximately 2% for developing schizophrenia, in comparison with the 0.5–1% risk in the general population. The risk increases to approximately 9% in first-degree relatives and to a concordance rate of up to 80% in monozygotic twins (Franzek and Beckmann 1998; Sullivan et al. 2003; Lichtenstein et al. 2006; Lichtenstein et al. 2009). The genetic liability is estimated to be 64–81% (Cardno and Gottesman 2000) and the heterogeneous phenotypic characteristics indicate a non-Mendelian and complex mode of inheritance (Thibaut 2006; Lee et al. 2012a; Kendler 2015). Common alleles with a high frequency in the general population together with rare variants may contribute to the risk of the disease.

**Endophenotypes**

Attempts have been made to deconstruct the complexity of the genetic underpinnings of schizophrenia by using more elementary measurable phenotypes associated with the disease (called intermediate phenotypes or endophenotypes; Ferrarelli 2013). To meet endophenotype criteria, candidate markers must have the following characteristics: (1) heritable; (2) relatively state-independent and stable over time; (3) associated with the illness (at least in part because of shared genetic influences); and (4) found in both affected and unaffected family members at a higher rate than in the general population (Gottesman and Gould 2003; Glahn et al. 2014). In the context of low correlation between phenotype and genotype in schizophrenia, these quantitative measurements could provide intermediate links between genetic variations and clinical phenotypes. Some neuropsychological endophenotypes have been widely studied in schizophrenia (Swerdlow et al. 2015; Thibaut et al. 2015) and seem promising for both diagnosis (Louchart-de la Chapelle et al. 2005; Thibaut et al. 2015) and measurement of functional outcome or response to treatment (Arfken et al. 2009; Light et al. 2012). Among neurophysiological endophenotypes, the largest effect sizes (with Cohen’s d effect size >1.5) were reported in schizophrenia patients for neuromotor deviation, auditory-evoked P50 paradigms, functional magnetic resonance imaging (fMRI) activation during the 2-back task, the Continuous Performance Test (CPT) and oculomotor-delayed response (Allen et al. 2009). Smaller effect sizes (Cohen’s d > 1) were reported by Light et al. (2012) for immediate verbal recall (California Verbal Learning Test-II, CVLT-II; List A), working memory (Letter Number Span, LNS; Wisconsin perseverative responses), mismatch negativity (MMN) and P300a amplitudes (associated with the automatic “Orienting Reflex”). Medium- to large-effect-size deficits have been reported in schizophrenia patients for the CPT, prepulse inhibition (PPI) of the acoustic startle reflex, the amplitude of MMN and P300 event-related potentials (Swerdlow et al. 2015). Deficits have also been described in first-degree relatives for LNS, CVLT, P50 suppression, N100 event-related potential amplitude, smooth pursuit eye movements (SPEM) and antisaccade performance (Louchart-de la Chapelle et al. 2005; Swerdlow et al. 2015). Some deficits, such as those observed with the PPI and P50 paradigms, may be sensitive to second-generation antipsychotic effects, so treatment with these antipsychotics needs to be controlled in analyses (Yee et al. 1998; Light et al. 2000).

In general, these measurements differ significantly between groups, are robust, reliable and state independent (except for P300 amplitude) and can be used as neurophysiological endophenotypes for genetic studies (Light et al. 2012; Swerdlow et al. 2015; Thibaut et al. 2015).

**Cognition**

Neuropsychological deficits in schizophrenia have remained robust over time, despite changes in
assessment instruments and diagnostic criteria, and have been replicated in different regions of the world, regardless of linguistic and cultural differences (Schaefer et al. 2013). Schizophrenia is associated with impairments across a wide range of cognitive domains and in information processing. Factor analysis studies concluded that seven distinct cognitive factors were replicable across studies and represent fundamental dimensions of cognitive deficit in schizophrenia (Nuechterlein et al. 2004): Processing speed, Attention/vigilance, Working memory, Verbal learning and memory, Visual learning and memory, Reasoning and problem solving and Verbal comprehension; the first six factors were recommended for inclusion in the MATRICS Consensus Cognitive Battery (MCCB), the final version of which consists of 10 neuropsychological tests. In 2004, a seventh cognitive domain has been included in the NIMH-MATRICS: social cognition, because of its potential implication in functional outcome in schizophrenia (Nuechterlein et al. 2004). Indeed, data suggest that social cognition is a relatively unitary dimension that may serve as a mediator between neurocognitive deficits and functional outcome in schizophrenia.

However, there is still considerable uncertainty as to which is the best test to perform (Dickinson et al. 2007; Reichenberg and Harvey 2007; Bakkour et al. 2014); competing testing arrays include the NIMH-MATRICS Consensus Cognitive Battery (Green et al. 2004) and the CogState Schizophrenia Battery (Pietrzak et al. 2009). The domains’ better correlations with social skills observed by Lees et al. (2015), suggest that the MCCB performance may have an advantage for measuring cognition in relation to functional outcome (Lees et al. 2015).

Deficits in the CPT in schizophrenia were reliably detected across five Northern American sites (2,251 participants), are relatively independent of current symptom severity and are related to functional capacity (Nuechterlein et al. 2015).

The influence of gender effects on cognitive performance, especially verbal performance, is still under debate (Canuso and Pandina 2007). The correlation between cognitive impairment and symptom severity in schizophrenia is only modest (Dominguez Mde et al. 2009; Ventura et al. 2010), and neurocognitive functioning does not appear to impact medication adherence (Sendt et al. 2015). Verbal memory and social cognitive deficits appear, however, to be the more robust markers of worse clinical outcome in schizophrenia (Lepage et al. 2014). The domain of social cognition was found to have a stronger association with community functioning and mediated an indirect relationship between neurocognition and functional outcome (Fett et al. 2011; Schmidt et al. 2011). Cognitive function in schizophrenia patients appears to not necessarily follow a pattern of age-related decline, but deterioration accompanies and is related to: (1) onset of early symptoms and the first psychotic episode and then (2) follows natural aging at approximately age 65 years (Harvey 2014). The high-risk state of psychosis is associated with overall impairment in neurocognitive functioning and social cognition. Transition to psychosis appears to be particularly associated with deficits in verbal fluency and memory functioning (Fusar-Poli et al. 2012). Although impairment in verbal comprehension, scholastic achievement and verbal working memory are found among children at risk for schizophrenia (Dickson et al. 2014), predictive cognitive markers that discriminate acutely ill, stabilised or recovered patients from healthy individuals are still lacking (Saoud et al. 2000; Addington and Barbato 2012).

Cognitive impairment has been reported in bipolar disorders, particularly in patients with a history of psychotic symptoms (Tsitispa and Fountoulakis 2015). Whether a generalised deficit exists across a spectrum of psychotic disorders is less clear. In addition, in the context of a broad cognitive impairment it remains difficult to identify deficits in specific cognitive processes with distinct neurochemical or regional brain substrates and linkages to particular risk-associated genetic factors (Reilly and Sweeney 2014).

**Neuroimaging**

If schizophrenia has a biological basis, patients should show some characteristic structural and functional features that underlie the pathological process, and these features should be traceable by neuroimaging techniques. Indeed, a large proportion of schizophrenia patients have more or less pronounced abnormalities in various brain regions, including thinning of the frontal and temporal cortex (Hajima et al. 2013). However, it remains unclear whether these abnormalities are specific for schizophrenia and to what degree the findings of the vast majority of neuroimaging studies are clinically relevant (Sommer and Kahn 2015).

The so-called “hypofrontality” hypothesis (Weinberger & Berman 1988), which is in accordance with the dopamine (DA) hypothesis of evidence of decreased functional connectivity and impaired white matter integrity in frontal, temporal, thalamic and striatal regions in schizophrenia patients. The correspondence, i.e., the interregional coupling in neural activity associated with well-myelinated white matter pathways shown in controls was absent in schizophrenia.
patients. Such relations between function and structure in parietal, occipital and temporal cortices were not affected (Cocchi et al. 2014). The most characteristic change found in structural neuroimaging of schizophrenia patients is a reduction of grey matter volume, which tends to be more pronounced on the left side of the brain, especially in frontotemporal and limbic regions (Mueller et al. 2012; Fujiwara et al. 2015).

White matter changes and altered brain connectivity are considered to be related to the risk of developing schizophrenia (Sommer and Kahn 2015). Voxel-based morphometry (VBM) revealed a reduction in white matter in the frontal cortex and internal capsule bilaterally in schizophrenia patients compared to controls (Di et al. 2009), and diffusion tension imaging (DTI) showed a reduction in fractional anisotropy values, mainly in frontal and temporal deep white matter in the left hemisphere (Ellison-Wright and Bullmore 2009).

A multimodal approach to the structural and functional changes in schizophrenia may be optimal: a combination of fMRI, VBM and DTI revealed overlapping abnormalities (Mueller et al. 2012). Functional and structural connectivity changes have been found in schizophrenia patients compared with healthy controls (Figure 1). Resting-state functional connectivity density was decreased in schizophrenia patients in the bilateral sensorimotor cortices and the right occipital cortex (Zhuo et al. 2014). Altered structural integrity of white matter has been found in schizophrenia patients in various brain regions (frontal and temporal regions, cingulum bundles, uncinate fascicule, internal capsules and corpus callosum), suggesting impairment of

Figure 1. Multimodal imaging methods in schizophrenia. (A) Cortical resting-state functional MRI connectivity networks based on 1,000 healthy subjects (Yeo et al. 2011). The seven networks with similar patterns of connectivity found by Yeo et al. were used and projected on the Conte69 standard MRI template. The black lines show cortical brain regions that are frequently structurally and/or functionally impaired in schizophrenia. The left hemisphere is more often affected. Visualisation was done using workbench (http://www.humanconnectome.org/software/connectome-workbench.html). (B) Diffusion tensor fractional anisotropy (FA) shown as greyscale diffusion metrics (left) as colour-coded principal direction of diffusion. Left-Right, red; anterior-posterior, green; inferior-superior, blue. Data from one sample schizophrenic patient of an ongoing study (64 diffusion directions, $2 \times 2 \times 2$ mm spatial resolution). (C) At group-level the FA metrics of every single subject are projected on a white matter “skeleton” and differences between schizophrenic patients and healthy subjects can be displayed (used software: FSL: http://www.fmrib.ox.ac.uk/fsl, own data of a study with $n = 22$ schizophrenic patients and age- and gender-matched subjects; red-yellow, significant clusters of decreased FA values in schizophrenic patients compared with healthy controls; dark-blue, left inferior fronto-occipital fasciculus; light-blue, left inferior longitudinal fasciculus). (This figure can be found in full colour in the online version of the article.)
multiple brain circuits in schizophrenia (Wheeler and Voineskos 2014).

Initial findings indicate that associations may exist between the observed structural changes and some characteristic clinical features of schizophrenia. Deficits in social cognition in schizophrenia, e.g., reduced recognition of facial emotions, were associated with structural abnormalities in various brain regions (Matsukawa and Murai 2013; Fujiwara et al. 2015). Motor abnormalities have been found in schizophrenia patients and are supported by neuroimaging studies of the cerebellum which revealed certain topographic changes in both schizophrenia patients and patients in at-risk states (Bernard and Mittal 2014). Also, parkinsonism has been associated with hyperechogenicity of the substantia nigra, both in chronic untreated schizophrenia patients and their relatives (Kamis et al. 2015).

A recent meta-analysis indicated that multivariate pattern analysis (MVPA) (Figure 2) allows schizophrenia patients to be differentiated from healthy controls with 80% accuracy on the basis structural neuroimaging data (Kambeitz et al. 2015). Another potential application of MVPA is in the field of differential diagnostics. In fact, because of the substantial symptomatic heterogeneity within and overlap across different psychiatric diagnoses, differential diagnostics might represent the greatest clinical challenge in everyday care. Thus MRI-based differential diagnosis may develop into one of the most promising applications of MVPA. To date, several studies have investigated the reliability of MVPA techniques in separating different psychiatric populations at the single-patient level with fMRI and structural MRI (sMRI) data. Classification accuracies of 67 and 80% have been reported for the differentiation between depressive and bipolar patients on the basis of MVPA of fMRI data (Mourao-Miranda et al. 2012; Grotegerd et al. 2014). One study reported only relatively low accuracies for MVPA of sMRI data (Serpa et al. 2014), but a more recent study demonstrated 79% classification accuracy for the differentiation between patients with unipolar and bipolar depression (Redlich et al. 2014), and another study showed 88% accuracy for the differentiation between bipolar patients and patients with schizophrenia (Schnack et al. 2014). One DTI study reported accuracies of 72–88% when MVPA was used to differentiate healthy women from female schizophrenia patients (Ota et al. 2012). Another recently published study used near-infrared spectroscopy to differentiate between depressed, bipolar and schizophrenia patients (Takizawa et al. 2014) and measured classification accuracies of 75% for depressed and 86% for bipolar and schizophrenia patients. Moreover, MVPA of imaging data has been applied to differentiate between different types of dementia, e.g., to separate Alzheimer’s dementia from frontotemporal dementia (Davatzikos et al. 2008). Although findings point to a promising potential application of MVPA of imaging data in the field of psychiatry, a multitude of methodological factors as well as characteristics of the investigated patient samples could likely moderate the success of MVPA-based models in classifying patients; consequently, future studies should address these factors in order to evaluate the potential of MVPA to inform everyday diagnostic workflows.

Functional imaging can identify abnormal brain function in schizophrenia patients. For instance, altered activation of the prefrontal cortex (PFC) and especially the dorsolateral prefrontal cortex (DLPFC) during working memory tasks is the most frequently documented finding in schizophrenia patients. When schizophrenia patients are compared with healthy controls, some parts of the PFC show a reduced blood oxygen level-dependent fMRI contrast and others show an increased one (Minzenberg et al. 2009). Altered activation of the PFC during working memory tasks and tasks that require “cognitive control” can be found in healthy first-degree relatives of schizophrenia patients (MacDonald et al. 2009). Recently, a growing number

Figure 2. Schematic illustration of the generation of neuroimaging-based multivariate classification models by using a cross-validation approach. Initially the data-set is separated into test and training data. In order to reduce the dimensionality of the data and to restrict the analysis to the most informative variables, a feature selection procedure might be implemented. In the model generation step a multivariate classification model is fitted to the training data. The models are then applied to the test data to estimate the classification performance of the model. In a cross-validation procedure all previous steps are repeated multiple times with different permutations of training-and test data to achieve a reliable estimate of the models’ accuracy.

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<th>Neuroimaging data-set</th>
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of studies have documented another functional impairment in schizophrenia patients: deactivation of the default mode network when switching to a cognitive task. This applies in particular to the medial frontal cortex, which is one of the main constituents of this network (Buckner et al. 2008). Studies in resting-state functional connectivity seem to underpin these findings (Greicius 2008). Functional connectivity is defined as the temporal dependency of neuronal activation patterns of anatomically separated brain regions. However, examination of the default mode network in relatives of schizophrenia patients has so far been limited and findings have differed (Whitfield-Gabrieli et al. 2010). Future work in the field needs to continue to integrate brain imaging information across structural and functional modalities. In the future, these techniques could contribute to the understanding of altered brain plasticity and may lead to a better understanding of pathophysiologically aspects of schizophrenia.

Research with positron emission tomography (PET) has validated the link between dopaminergic abnormalities, schizophrenia, schizophrenia symptoms and the risk for disease (Kasper et al. 2002). Furthermore, because PET allows changes to be elucidated at a molecular level it facilitates investigations into which cellular and functional components of a neurotransmitter system are affected. The most consistent PET evidence in schizophrenia has been found in the dopaminergic system. First, schizophrenia has been repeatedly associated with an increase in striatal $^{[18F]}$DOPA and $^{[11C]}$DOPA uptake, which reflects an increase in presynaptic dopaminergic synthesis (Fusar-Poli and Meyer-Lindenberg 2013b). Elevated dopaminergic synthesis has been demonstrated in cohorts of patients who had, at least in part, previously or currently medicated (Lindstrom et al. 1999; Meyer-Lindenberg et al. 2002; McGowan et al. 2004; Kumakura et al. 2007; Howes et al. 2009), and neuroleptic-naive patients (Hietala et al. 1995; Hietala et al. 1999), and has been shown to correlate with specific psychotic symptoms (McGowan et al. 2004). Persons at risk for developing schizophrenia also exhibit increased $^{[18F]}$DOPA uptake, which may correlate with the severity of prodromal symptoms (Howes et al. 2009). It has been demonstrated that evaluation of striatal $^{[18F]}$DOPA uptake allows for classification of schizophrenia patients and controls with approximately 60–90% sensitivity and specificity, depending on the quantification method used (Bose et al. 2008). In addition, dopamine transporter (DAT) distribution also reflects presynaptic dopaminergic function and can be interpreted as an index of dopaminergic cell density. DAT ligand-binding results are, however, more inconsistent: Studies show evidence of increased $^{[11C]}$PE2I thalamic (Arakawa et al. 2009) and reduced $^{[18F]}$FCFT striatal binding (Laakso et al. 2001). Symmetry of caudate DAT binding has been suggested to differ between schizophrenia patients and healthy controls (Laakso et al. 2000). Nevertheless, a meta-analysis of 13 PET studies on DAT density that included 202 patients regardless of prior or current antipsychotic medication made a strong case for a lack of change in DAT distribution in schizophrenia (Fusar-Poli and Meyer-Lindenberg 2013a). Furthermore, although extensively investigated, PET studies on DA receptor levels – in particular the D2 receptor – are similarly inconsistent (Suhara et al. 2002; Talvik et al. 2003; Yasuno et al. 2004) Buchsbaum et al. 2006; Talvik et al. 2006; Kessler et al. 2009; Kegeles et al. 2010; Suridjan et al. 2013). This inconsistency results in part from interaction between endogenous DA and the applied radioligand, and also from various factors that modulate DA levels. However, imaging of D2 and D3 receptors with radioligands such as $^{[11C]}$raclopride (Farde et al. 1986; Verhoeff et al. 2002), $^{[18F]}$fallypride (Mukherjee et al. 2002; Cropley et al. 2008) and $^{[11C]}$PHNO (Willeit et al. 2006; Caravaggio et al. 2014) also allows for indirect assessment of DA levels in response to dynamic conditions (Figure 3). Basal DA levels, which can be assessed by DA depletion and a resulting increase in receptor availability, are significantly higher in schizophrenia patients: receptor availability increases by about 15% in patients versus 10% in controls (Kegeles et al. 2010). Schizophrenia has also been repeatedly and consistently associated with increased dopaminergic reactivity both to amphetamine (Breier et al. 1997) and psychosocial stress (Mizrahi et al. 2012). For example, amphetamine application in schizophrenia patients results in a significantly higher reduction in $^{[11C]}$raclopride binding (which represents DA release) in patients (>20%) than in controls (about 15%). These results were not affected by previous antipsychotic treatment (Breier et al. 1997). Single-photon emission-computed tomography (SPECT) studies have shown a tracer reduction that is twice as high in patients than in controls (Laruelle et al. 1996). This increased reactivity to amphetamine challenge in schizophrenia patients has been replicated several times in PET and SPECT studies and might be one of the most promising molecular neuroimaging markers in schizophrenia. Such modulation of dopaminergic function is likely to reflect a neurobiological correlate of risk for schizophrenic symptoms. Perhaps most compellingly, prodromal and clinically high-risk groups also show changes in these dopaminergic PET parameters (Hirvonen et al. 2005;
Lee et al. 2008; Howes et al. 2009; Brunelin et al. 2010). However, as of yet, studies in at-risk patients lack sufficient power for assessment of the clinical applicability of these findings.

PET studies on changes to the serotonergic system are far less extensive and less conclusive. 5-HT$_{2A}$ represents an important target because of its role in the pharmacodynamics of atypical neuroleptics. Changes to 5-HT$_{2A}$ receptor distribution have been shown in a range of reports, although with widely inconclusive results (Erritzoe et al. 2008; Hurlemann et al. 2008; Rasmussen et al. 2010). In addition, changes to the 5-HT$_{1A}$ receptor have been demonstrated with PET in some studies (Tauscher et al. 2002), but not in others (Frankle et al. 2006). The inconsistency in serotonergic PET findings in schizophrenia may be due in part to the heterogeneity of the schizophrenia patient groups investigated in these studies and high variability within the serotonergic system, in contrast to more consistent findings in dopaminergic function in this patient group.

In summary, changes to DA synthesis and basal and challenge-related endogenous DA levels represent the most consistent and therefore convincing PET evidence in schizophrenia, especially the strongly increased DA levels after amphetamine challenge. Although data on changes to the dopaminergic system in prodromal and at-risk states show great potential for clinical relevance, sensitivity and specificity need to be investigated in larger patient groups. Currently, PET studies have yet to reveal consistent data on changes to the serotonergic system.

A number of magnetic resonance spectroscopy (MRS) studies have demonstrated alterations in the concentrations of glutamate, glutamine (a precursor of glutamate) and Glx (a combination of both amino acids) in antipsychotic-naïve, antipsychotic-free or medicated schizophrenia patients (Poels et al. 2014a, 2014b; Salavati et al. 2015). These studies revealed elevated Glx levels in the medial prefrontal cortex (mPFC), parietal, occipital, anterior cingulate, thalamus and basal ganglia regions; elevated levels of glutamine in the mPFC, thalamus and anterior cingulate cortex; elevated levels of glutamate in the basal ganglia; decreased levels of glutamate in the thalamus; and no differences or unclear findings regarding glutamatergic metabolites in the DLFPC and temporal and cerebellum regions (Salavati et al. 2015). Some studies (de la Fuente-Sandoval et al. 2011; Kegeles et al. 2012; de la Fuente-Sandoval et al. 2015) showed elevated levels of glutamate (or Glx) in the prodromal state and in unmedicated schizophrenia patients. A recent MRS study showed an increase of glutamate in the frontal and anterior cingulate cortex of antipsychotic-naïve patients with first-episode psychosis, and found a positive correlation between glutamate and membrane lipids (phosphomonooesters and phosphodiesters) in the patients (Smesny et al. 2015). Furthermore, another...
recent MRS study showed an increase of glutamate in the associative striatum of antipsychotic-naïve patients with first-episode psychosis and found positive correlations between glutamate and myo-inositol (or choline) in the antipsychotic-naïve patients with first-episode psychosis (Plitman et al. 2015). Given the role of myo-inositol and choline in the glial cells, dysregulation of glial function is likely to result in the disruption of glutamatergic neurotransmission, which may play a role in the psychosis in the first-episode patients. Overall, glutamate levels are somewhat elevated in early-phase, drug-free patients and decrease after treatment (Poels et al. 2014a, 2014b). In 2005, we reported an elevated ratio of glutamate to glutamine in cerebrospinal fluid (CSF) samples of first-episode and drug-naïve patients with schizophrenia (Hashimoto et al. 2005a), suggesting an abnormality of the glutamine–glutamate cycle in neuron–glia communication in schizophrenia.

Glutathione (GSH; L-glutamyl-L-cysteinyl-glycine), the most abundant thiol in mammalian cells that is present at concentrations of up to 12 mM, is a nucleophilic scavenger and an enzyme-catalysed antioxidant. Glutathione potentiates the N-methyl-D-aspartate (NMDA) receptor response to glutamate by acting at the redox modulatory site(s) (Hashimoto 2014b). Do et al. (2000) reported that the CSF levels of GSH in drug-free schizophrenia patients were significantly (27%) lower than in controls. A $1^\text{H}$-MRS study demonstrated that the levels of GSH in the mPFC of schizophrenia patients were 52% lower than in controls (Do et al. 2000). Subsequently, Matsuzawa et al. (2008) found a negative correlation between GSH levels in the prefrontal cortex and the severity of negative symptoms in schizophrenia patients, although no differences in GSH levels were detected between the two groups (Matsuzawa et al. 2008). Currently, the MRS findings concerning GSH in the brain are inconsistent. The differences in sequences and spectral editing techniques and clinical factors (e.g., medication status, phase of illness) may contribute to the discrepancies in GSH concentration (Poels et al. 2014a). Interestingly, the antioxidant N-acetyl-L-cysteine, a precursor of GSH, has been reported to be effective in the treatment of schizophrenia (Berk et al. 2008). In summary, it is likely that oxidative stress associated with reduced GSH levels in the brain plays a crucial role in the pathophysiology of schizophrenia (Hashimoto 2014b).

**Neurogenetics**

In this section, we will present the most important genetic findings in neuropsychiatric diseases. Most of the GWA studies provide only small odds ratios and therefore are not suitable as biomarkers in a single individual. Nevertheless, copy number variation (CNV) studies provide very high odds ratios, assuming that these deletions or duplications will be accepted as biomarkers in the near future.

**GWA studies**

GWA studies represent a major development in human genetics. Technological progress currently allows up to 1,000,000 single nucleotide polymorphisms (SNPs) to be genotyped in one run. The first GWA study on schizophrenia was performed with 178 cases and 144 controls and showed an association of CSF2RA (colony-stimulating factor, receptor 2a); the second study found no genome-wide significance (Lencz et al. 2007; Sullivan et al. 2008). Both studies seemed underpowered to provide conclusive results. The first GWA study to deal with this problem performed an initial GWA on 479 cases and 2,937 controls; in a subsequent international survey in up to 6,829 cases and 9,897 controls the research group then examined loci that had surpassed $P < 10^{-5}$ (O’Donovan et al. 2008). Among 12 of these loci, three had strong independent support and the overall pattern of replication was unlikely to occur by chance. The association was strengthened for the top gene, ZNF804A, when bipolar disorder was included in the affected phenotype, a finding that challenged the traditional diagnostic boundaries and simultaneously reduced the specificity of ZNF804A as a biological marker for schizophrenia (O’Donovan et al. 2008). Within the SGENE+ consortium, a further GWA analysed 2,663 schizophrenia cases and 13,498 controls from eight European locations (Stefansson et al. 2009). The study combined findings from the top 1,500 markers with results for these same markers (or surrogates for them) from both the International Schizophrenia Consortium (ISC; 2,602 cases/2,885 controls) and the European-American portion of the Molecular Genetics of Schizophrenia (2,687 cases/2,656 controls) studies. The top markers were followed-up in 5,013 cases and 15,559 controls from four sets of...
additional samples from Europe, leading to the identification of three novel candidate schizophrenia loci: neurogranin, transcription factor 4 (TCF4) and the human leukocyte antigen (HLA) region (Stefansson et al. 2009). The HLA associations were also found in the large GWA by the ISC (International Schizophrenia Consortium et al. 2009), which studied 3,322 Europeans with schizophrenia and 3,587 controls, as well as by Shi et al. (2009).

Because sample size plays a crucial role in GWA studies, the Schizophrenia Psychiatric Genome-Wide Association Study Consortium (PGC) combines as many schizophrenia cases and controls as possible. In 2011, the PGC published results on 51,695 individuals of European ancestry. The combined analysis yielded non-replication in most of the earlier candidate regions but found genome-wide significant associations with schizophrenia for seven loci, five of which were new (1p21.3 [MIR137], 2q32.3 [PCGEM1], 8p23.2 [CSMD1], 8q21.3 [MMP16] and 10q24.32-q24.33 [CNNM2, NT5C2]) and only two of which had been implicated previously (6p21.32-p22.1 [TRIM26] and 18q21.2 [CCDC68, TCF4]). The strongest new finding was for rs1625579 within an intron of a putative primary transcript for MIR137 (microRNA 137), a known regulator of neuronal development. Four other schizophrenia loci that achieved genome-wide significance predicted targets of MIR137, suggesting MIR137-mediated dysregulation as a previously unknown aetiological mechanism in schizophrenia. In a joint analysis with a bipolar disorder sample (16,374 affected individuals and 14,044 controls), three loci reached genome-wide significance (Lee et al. 2012b): CACNA1C (rs4765905), ANK3 (rs10994359) and the ITIH3-ITIH4 region (rs2239547).

Another large GWA study, with 5,001 cases and 6,243 controls, was followed by a meta-analysis with previous schizophrenia GWA study data (8,832 cases and 6,243 controls, was followed by a meta-analysis with previous schizophrenia GWA study data (8,832 cases and 6,243 controls, was followed by a meta-analysis with previous schizophrenia GWA study data (8,832 cases and 6,243 controls, was followed by a meta-analysis with previous schizophrenia GWA study data (8,832 cases and 6,243 controls, was followed by a meta-analysis with previous schizophrenia GWA study data (8,832 cases and 6,243 controls, was followed by a meta-analysis with previous schizophrenia GWA study data (8,832 cases and 6,243 controls). The combined analysis yielded genome-wide significant associations with schizophrenia for seven loci, five of which were new (1p21.3 [MIR137], 2q32.3 [PCGEM1], 8p23.2 [CSMD1], 8q21.3 [MMP16] and 10q24.32-q24.33 [CNNM2, NT5C2]) and only two of which had been implicated previously (6p21.32-p22.1 [TRIM26] and 18q21.2 [CCDC68, TCF4]). The strongest new finding was for rs1625579 within an intron of a putative primary transcript for MIR137 (microRNA 137), a known regulator of neuronal development. Four other schizophrenia loci that achieved genome-wide significance predicted targets of MIR137, suggesting MIR137-mediated dysregulation as a previously unknown aetiological mechanism in schizophrenia. In a joint analysis with a bipolar disorder sample (16,374 affected individuals and 14,044 controls), three loci reached genome-wide significance (Lee et al. 2012b): CACNA1C (rs4765905), ANK3 (rs10994359) and the ITIH3-ITIH4 region (rs2239547).

Notable associations relevant to the glutamate hypothesis of schizophrenia include the four genes GRM3 (metabotropic glutamate receptor 3), GRIN2A (NMDA receptor subunit GluN2A), GRIA1 (GluA1 subunit of AMPA receptor) and SRR (serine racemase) (Table 1) (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014)( Figure 4). NMDA receptor binding was increased in the putamen of schizophrenia patients compared to controls (Kornhuber et al. 1989), possibly to compensate for low tissue levels of glutamate and NMDA (Errico et al. 2013). Protein levels of mGluR3, but not mGluR2, were decreased in the DLPFC of schizophrenia compared to controls (Ghose et al. 2009). Expression of GluN1, GluN2A and GluN2B was downregulated in the DLPFC of schizophrenia patients compared to controls and correlated to lower levels of D-aspartate and NMDA in schizophrenia patients (Errico et al. 2013), confirming the NMDA receptor hypofunction hypothesis in schizophrenia. In short-term habituation, Gria1 knock-out mice showed sensitisation and selective impairment, both of which are relevant to schizophrenia (Barkus et al. 2014). SRR is an enzyme that generates d-serine, an endogenous obligatory coagonist of NMDA receptors, from l-serine (Hashimoto
Figure 4. P values of 108 L,D-independent genome-wide significant loci identified in the largest GWA study in schizophrenia conducted by the Psychiatric Genomics Consortium. P values of associated regions correspond to the smallest P value of all markers in that region. Highlighted are regions and selected associated genes in relevant pathways. It must be stressed that “association only implies the existence of one or more risk variant at the associated locus rather than that a specific gene is responsible for the association” (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014).

Table I. Glutamate-related genes identified in the most recent and largest GWA study by the Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal localisation</th>
<th>Biological function</th>
<th>Alteration in schizophrenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRM3</td>
<td>7q21.1-q21.2</td>
<td>Encodes the metabotropic glutamate receptor 3 (mGluR3). mGluR3 is predominantly localised in glia cells. N-acetylaspartylglutamate (NAAG) is an endogenous agonist at mGluR3.</td>
<td>Reduction in mGluR3 protein in the DLPFC in schizophrenia, with no changes in mGluR2 protein (Ghose et al. 2009).</td>
</tr>
<tr>
<td>GRIN2A</td>
<td>16p13.2</td>
<td>Encodes the NMDA receptor subunit GluN2A, a key modulator of synaptic plasticity. NMDA receptor antagonists such as phencyclidine and ketamine cause schizophrenia-like psychotic symptoms (e.g., positive and negative symptoms and cognitive impairment) in humans.</td>
<td>NMDA receptor binding was increased in the putamen of patients with schizophrenia (Kornhuber et al. 1989). Tissue levels of GluN1, Glu2A and GluN2B in the prefrontal cortex with schizophrenia were lower than those of controls (Errico et al. 2013). Furthermore, tissue levels of d-aspartate and NMDA in the prefrontal cortex of schizophrenia were also lower than those of controls (Errico et al. 2013).</td>
</tr>
<tr>
<td>GRIA1</td>
<td>5q31.1</td>
<td>Encodes the GluA1 subunit of AMPA receptor that mediates fast synaptic transmission.</td>
<td>Gria1 knock-out (KO) mice showed selective impairments in short-term habituation and also sensitisation (Barkus et al. 2014). These deficits are relevant to patients with schizophrenia.</td>
</tr>
<tr>
<td>SRR</td>
<td>17p13</td>
<td>Encodes serine racemase (SRR), which synthesises d-serine from l-serine in the neuron. d-serine is an endogenous obligatory co-agonist of the NMDA receptor and binds to the glycine modulatory site of the GluN1 subunit in the NMDA receptor.</td>
<td>Decreased levels of d-serine in blood and CSF from schizophrenia patients (Hashimoto et al. 2003; 2005a; Bendikov et al. 2007). D-serine is effective in the treatment of patients with schizophrenia (Tsai and Lin, 2010).</td>
</tr>
</tbody>
</table>
et al. 2013; Hashimoto 2014b). Previously, reduced levels of \(\alpha\)-serine and a reduced ratio of \(\alpha\)-serine to total serine were reported in both the blood and CSF of schizophrenia patients (Hashimoto et al. 2003; Hashimoto et al. 2005b; Bendikov et al. 2007). In addition, \(\alpha\)-serine was shown to be effective in the treatment of several symptoms of schizophrenia (Tsai and Lin 2010). Therefore, decreased \(\alpha\)-serine levels may contribute to the NMDA receptor hypofunction in schizophrenia, and \(\alpha\)-serine treatment may be more effective in patients with decreased \(\alpha\)-serine levels. However, it has to be noted that beside well-known dopaminergic and serotonergic neurotransmitters, antipsychotic treatment may also influence the glutamatergic system (Zink et al. 2014).

In summary, from GWA studies it seems that the association between the HLA region and schizophrenia is the most robust and replicated result from GWA studies. Other polymorphisms suggest a glutamatergic dysfunction in schizophrenia and also in some pathways involved in neurodevelopment, but until now they do not constitute risk factors, even at the population level. In addition to common variants, rare structural variants have been associated with schizophrenia risk.

Identification of rare risk variants before GWA studies

The fact that a Mendelian mode of inheritance could not be identified for schizophrenia indicated that either no or only a very few rare mutations such as the 22q11.2 microdeletion or the balanced chromosomal translocation that disrupts the DISC1 gene (1;11)(q42.1;q14.3) play a significant aetiological role in schizophrenia. The situation changed with the detection in GWA studies of microdeletions and microduplications, which may significantly increase the risk of schizophrenia in a given person (Stefansson et al. 2008).

The disruption of genes by balanced translocations detectable at the microscopic level has played an important part in the discovery of many common Mendelian disorder genes. The Disrupted-in-Schizophrenia-1 (DISC1) gene is an example of such a disruption. DISC1 was identified via a balanced translocation at 1:11(q42; q14.3) and linked to both schizophrenia and depressive disorders in a Scottish family (St Clair et al. 1990). It is a highly pleiotropic gene with effects on cellular proliferation, migration, dendritogenesis, synaptogenesis and intracellular signalling and appears to be important for both the developing and adult brain (Porteous et al. 2014; Randall et al. 2014).

A microdeletion at 22q11.2 is responsible for velo-cardiofacial syndrome (VCFS), also known as DiGeorge syndrome. In 1992, besides the characteristic dysmorphic and congenital symptoms, Shprintzen et al. (1992) first described the high risk of schizophrenia-like psychotic symptoms in people with this syndrome (in about 30% of patients). The hope that this deletion could serve as a model for schizophrenia resulted in a series of animal models and cell culture studies. However, it is still unclear which of the genes in this deletion region are responsible for the predisposition of people with VCFS to neurocognitive deficits and psychosis. Catechol-O-methyltransferase (COMT) and ProDH genes may be involved in mental retardation and psychosis (Raux et al. 2007). For instance, the deletion region contains genes involved in brain function and neurodevelopment, including COMT, ProDH, TBX1, GNB1L and more (Jacquet et al. 2002; Squarcione et al. 2013; Schneider et al. 2014).

CNVs through GWA studies

With the completion of the Human Genome Project, the rapid development of new whole-genome scanning technologies has started to fill the resolution gap between traditional cytogenetic analysis (>2 Mb) and mutation analysis by DNA sequencing (<1 kb). As a result, a large number of previously undetected variations in the human genome have been revealed. One of the most intriguing types of variation, whose extent was previously unappreciated, are CNVs, the loss or gain (not only deletion or duplication as the author believes) of genomic DNA with a size larger than 1 kb (Grayton et al. 2012).

A first family-based study suggested that de novo CNVs are significantly more common in sporadic cases than in controls or familial cases of schizophrenia, accounting for approximately 10% of the sporadic cases (Xu et al. 2008), whereas an independent case–control study found novel deletions and duplications of genes in 5% of controls versus 15% of cases and 20% of young-onset childhood-onset cases (Walsh et al. 2008). Both studies were statistically underpowered to prove the involvement of any specific CNV.

The SGeneþ consortium studied a population-based sample by analysing 9,878 transmissions from parents to offspring (Stefansson et al. 2008). Sixty-six de novo CNVs were identified and tested for association in a sample of 1,433 schizophrenia cases and 33,250 controls; among these, three deletions (1q21.1: 0.23 and 0.02%, odds ratio [OR] = 14.83; 15q11.2: OR = 2.73; and 15q13.3: 0.17 and 0.02%, OR = 11.54; schizophrenia patients vs. controls, respectively) showed nominal
association in the first sample and were followed-up in a second sample of 3,285 cases and 7,951 controls. In a genome-wide survey of rare CNVs in 3,391 patients with schizophrenia and 3,181 controls (International Schizophrenia Consortium 2008), the total number of CNVs >100 kb in length and observed in less than 1% of the sample was increased 1.15-fold in patients with schizophrenia in comparison with controls. Associations for 15q13.3, 1q21.1 and 15q11.2 have been replicated by different studies, including meta-analyses (Kirov et al. 2009a; Levinson et al. 2011; Rees et al. 2014b).

Deletions within the neurexin 1 gene (NRXN1; 2p16.3), previously associated with autism, have also been reported in two families with schizophrenia (Kirov et al. 2008; Walsh et al. 2008). The SGENE+ consortium examined NRXN1 for CNVs in 2,977 schizophrenia patients and 33,746 controls. CNVs that disrupt exons were significantly enriched in cases (0.24% vs. 0.015% in controls) with OR =8.97 showing that NRXN1 deletions confer risk of schizophrenia (Rujescu et al. 2009). A meta-analysis of 8,789 schizophrenia patients and 42,054 controls showed a frequency of 0.19% of deletions (>100 kb) in patients and 0.04% in controls (OR =4.78, 95% CI 2.44–9.37). Odds ratios were even higher for deletions (>100 kb) that disrupt exons (OR =7.44, 95% CI 3.22–17.18) (Kirov et al. 2009b).

Further CNVs have been associated with schizophrenia, such as the 3q29 microdeletion (Mulle et al. 2010; Levinson et al. 2011), which showed the highest OR in a meta-analysis (OR =57.65, 95% CI 7.58–438.44) (Rees et al. 2014b). CNVs at 16p11.2 (Guha et al. 2013), 17p12 (Kirov et al. 2009a) and 17q12 (Moreno-De-Luca et al. 2010) were confirmed in a recent meta-analysis (Rees et al. 2014b).

Further studies added new deletions at DLG2 (discs large gene2, a member of the family of membrane-associated guanylate kinases), which is a component of the postsynaptic density, and at EHMT1, a histone methyl transferase known to directly regulate DLG family members (Kirov et al. 2012).

Besides deletions, several studies have provided evidence for an increased frequency of duplications and genomic gains in schizophrenia at the following locations: 1q21.1 (Levinson et al. 2011), 7q11.23 (genomic region of Williams-Beuren syndrome; Kirov et al. 2012; Mulle et al. 2014) and 7q36.3, which involves the neuropeptide receptor gene VIPR2 (Levinson et al. 2011; Vacic et al. 2011). Although the locus at 7q36.3 initially was not found to be significant, it showed a significant association in the meta-analysis (Rees et al. 2014b).

Duplications associated with schizophrenia are seen also at other locations. These duplications include 15q11-q13 (Ingason et al. 2011a) and 16p13.11 (Ingason et al. 2011b; Rees et al. 2014b), both of which were confirmed in a meta-analysis by Rees et al. (2014b). A duplication identified at 16p11.2 (McCarthy et al. 2009; Levinson et al. 2011; Steinberg et al. 2014) showed the strongest association in a different meta-analysis (Rees et al. 2014a).

It is notable that a number of CNVs identified as enriched in psychosis have also been identified as enriched in other neurodevelopmental and neurological disorders, including mental retardation, autism and seizures (Grayton et al. 2012; Grozeva et al. 2012). 16p11 and 3q29 deletions have also been reported in people with bipolar disorder (Lee et al. 2012b).

In summary, associations for 15q13.3 and 1q21.1 microdeletions have been replicated by different studies and have been reported in schizophrenia patients without mental retardation. Both microdeletions may significantly increase the risk for schizophrenia (about 15 times) in a given person. These rare and de novo mutations may be transmitted but their mode of inheritance remains poorly understood. In addition, the same CNVs may be associated with different phenotypic expressions (e.g., bipolar disorder, schizophrenia, autism, mental retardation and cranial size, even in the same families). They may also interact with other rare or common variants or with epigenetics, which may contribute to different phenotypic expressions.

**Next-generation sequencing**

Next-generation sequencing, a result of technological progress, is a recent breakthrough in genetics. Also, high-throughput sequencing has become available for both exome and whole-genome sequencing. These techniques can be applied in case–control studies or the detection of de novo mutations by sequencing both unaffected parents and the affected child (triplets) or families with high loading of schizophrenia subtypes (Stöber et al. 2009). However, sequencing may generate some data with false-positive variants and therefore studies should be replicated in large samples. Some studies on sequencing cases and their unaffected parents have shown promising results, but these have not been replicated (Girard et al. 2011; Xu et al. 2011, 2012; Gulsuner et al. 2013; McCarthy et al. 2014). The largest sequencing project in schizophrenia was performed on 623 triads and found evidence for de novo mutations over-represented among glutamatergic postsynaptic proteins comprising activity-regulated cytoskeleton-associated protein and
N-methyl-D-aspartate receptor (NMDAR) complexes (Fromer et al. 2014); this finding is supported by the largest exome-sequencing study so far performed with 2,536 schizophrenia cases and 2,543 controls (Purcell et al. 2014). There is hope that all these new technologies will help to dissect the heritability of schizophrenia and provide further insight into the pathophysiology of this disease.

**Associations between endophenotypes and susceptibility genes**

In contrast to GWA and CNV studies on the phenotype schizophrenia, the findings on endophenotypes are less convincing, although several endophenotypes have been linked to genes that are potentially consistent with the pathophysiological models for schizophrenia. Deficits in sensory-motor gating in schizophrenia patients have been linked to septohippocampal cholinergic activity (involving the alpha 7 subunit of nicotinic receptors). Promoter variants (in the alpha 7 gene) or variants located in the alpha 7-like gene were associated with P50 inhibition deficits but not with schizophrenia (Leonard and Freedman 2006; Raux et al. 2007; Cabranes et al. 2013; Liu et al. 2013). In addition, polymorphic sites at the TCF4 and the DAT 1 and 2 genes (DRD1, DRD2) may interact with smoking in the modulation of sensory gating (Knott et al. 2010; Millar et al. 2011; Quednow et al. 2012).

The COMT gene (22q11) may also be considered as a candidate gene for disturbances in sensory gating, PPI or SPEM in schizophrenia because of its physiological effects on prefrontal functions, including degradation of DA. The PPI index revealed an association between pulse latency and the Val158Met COMT polymorphism (Liu et al. 2013). Considering the evidence accumulated over time, there is little support for any association between COMT Val158Met and P50 gating deficits (Lu et al. 2007; Olincy et al. 2010; Shaikh et al. 2011; Liu et al. 2013) or SPEM disturbances (Haraldsson et al. 2009; Park et al. 2009; Rybakowski et al. 2002). The antisaccade latency may be influenced by the COMT gene (Haraldsson et al. 2010b), but in men with schizotypal disorder no association is evident between the antisaccade paradigm measures and the COMT polymorphism (Stefanis et al. 2004). In contrast, other genes located in the 22q11 region, such as the zinc finger DHHC domain-containing protein 8 (ZDHHC8) or the Ran-binding protein (RANBP) RANBP1-h1t2, might have some influence on SPEM function in schizophrenia patients, as was shown in a Korean population (Shin et al. 2010; Cheong et al. 2011).

A lack of association was reported between NRG1 and SPEM disturbances or P50 deficits in schizophrenia (Haraldsson et al. 2010a; Schmechtig et al. 2010; Shaikh et al. 2011; Kim et al. 2012) or the receptor tyrosine-protein kinase erbB-4 (ERBB4) gene (Bae et al. 2012). In contrast, NRG1 rs3924999 may affect spatial accuracy on the antisaccade task (Schmechtig et al. 2010).

Several genes have been associated with the auditory oddball P300 measures. COMT has been associated with amplitude (Gallinat et al. 2003; Golimbet et al. 2006) and NRG1 with latency (Kang et al. 2012) and a significant association was reported between the P300 amplitude elicited by novel sounds (P3b component) and ZNF804A (Del Re et al. 2014).

**Modifier genes**

Genetic factors that influence symptom dimensions without necessarily conferring risk for the disease have been reported as modifier genes. Edwards et al. (2015) reported associations between common genes and negative or positive symptoms of schizophrenia; notably, NKAIN2 and NRG1 were associated with negative and positive symptoms, respectively. However, no single marker has met the genome-wide significance threshold.

Some authors have reported associations between the total number of base pairs affected by copy number deletions or duplications genome wide and treatment resistance, clinical characteristics or cognitive abilities of schizophrenia patients, although the odds ratios are close to 1 (Yeo et al. 2013; Martin et al. 2014; Martin and Mowry 2015).

**Summary**

Genetic studies should also consider the potential for interactions between multiple loci at the same or different genes (epistasis). The genes identified so far from traditional mapping, CNV analysis and other strategies point to several biological pathways, such as the integrity of the synapse, the wnt/GSK3β signalling pathway (DISC1, TCF4, etc.), GRIK4 or NPAR5 (which are involved in neurogenesis in the brain), glutamatergic signalling (NRG1, DPNBP1, ProDH, L-amino acid oxidase activator, etc.), the dopaminergic system (COMT, DRD3, CHRNA7 indirectly), and the major histocompatibility complex. These findings are consistent with current developmental and biological hypotheses about schizophrenia. At the individual level, these susceptibility genes may not be helpful to predict the risk for schizophrenia (for more on this topic, see also the
guidelines of the International Society for Psychiatric Genetics www.ispg.org). Common variants (nucleotidic variations) increase the risk for schizophrenia slightly (OR ≤1.2), in contrast to some rare variants that may increase the risk significantly (e.g., OR = 15 for some microdeletions that are found in less than 5% of schizophrenia patients).

Endophenotypes are helpful to disentangle the complexity of the genetic underpinnings of schizophrenia by using measurable, heritable and simpler phenotypes associated with the disease. Also, additional risk genes can be identified in the heterogeneous populations of schizophrenia patients through strategies aimed at refining the phenotype that are not constrained by the current dichotomous view of functional psychoses. Schizophrenia, bipolar disorder and probably autism overlap substantially, at least in terms of polygenic risk. Some endophenotypes might also be useful as potential diagnostic marker tools (see Thibaut et al. 2015).

Neuroimaging studies that use newer methodologies such as functional or structural connectivity, or methods that assess reactions to pharmacological stimuli such as dopamine PET challenge studies, may be suitable to discover biomarkers of the disorder. However, schizophrenia is a heterogeneous and complex disorder and single biomarkers are missing because they do not capture the heterogeneity and complexity of this brain disorder (Atluri et al. 2013). Combinational biomarkers may involve genomic imaging, and new computer-based methods such as multivariate pattern analysis have been developed to identify complex biomarkers that allow for individualised differential diagnosis, as indexed by MRI-based, clinical and neurocognitive measures. Most importantly, this approach allows multivariate statistical models to be used to predict treatment response at the level of the individual participant and allows for the integration of different neuroimaging modalities (sMRI, resting-state MRI, fMRI) to build and compare both uni- and multimodal predictive models. Although biomarkers for schizophrenia await validation, the knowledge on candidate genomic and neuroimaging biomarkers is growing rapidly and research on this topic has the potential to identify psychiatric endophenotypes and increase knowledge on individual treatment response.

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References


Lee KW, Woon PS, Teo YY, Sim K. 2012a. Genome wide association studies (GWAS) and copy number variation (CNV) studies of the major psychoses: what have we learnt? Neurosci Biobehav Rev. 36:556–571.


Sommer IE, Kahn RS. 2015. The contribution of neuroimaging to understanding schizophrenia; past, present, and future. Schizophr Bull. 41:1–3.


