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REVIEW

The utility of biomarker discovery approaches for the detection of disease mechanisms in psychiatric disorders

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Schizophrenia remains an elusive multifaceted disorder with all evidence of its onset and aetiology pointing to a complex interplay of genetic, nutritional, environmental and developmental factors. Although several molecular and structural abnormalities have been reported for schizophrenia, no diagnostic test or other application of clinical use has yet emerged from this research. The heterogeneity of schizophrenia symptoms and its similarity to other psychiatric disorders, the accessibility of appropriate samples and the complexity of molecular alterations have greatly slowed down research. Biomarker discovery approaches should ultimately facilitate objective diagnosis, allow the identification of at-risk individuals, predict treatment success and revolutionize drug-discovery approaches. For psychiatric disorders, large sample numbers are necessary if disease-intrinsic alterations are to be detected in an environment of high biological variability. Only recent technological advances facilitate the profiling of proteins and metabolites of large sample numbers. These approaches promise to provide interesting insights into disease mechanisms, as they enable capturing the dynamic nature of disease-related alterations. By means of parallel profiling using a multi-omics approach, we aim to disentangle the complex nature of schizophrenia's aetiology. Here, we will outline how this system-based analysis approach can contribute to the discovery of disease mechanisms in schizophrenia and in turn other psychiatric disorders.
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Abbreviations: MALDI, matrix-assisted laser desorption ionisation; SELDI, surface-enhanced laser desorption ionisation; DIGE, differential in gel electrophoresis; ICAT, isotope-coded affinity tags; iTRAQ, isobaric tags for relative and absolute quantitation

Schizophrenia and the need for improved treatment approaches

Schizophrenia, bipolar disorder and severe clinical depression are a group of mental diseases, which often manifest themselves with psychotic states characterized by disruption of basic perceptual, cognitive, affective and judgmental processes. Individuals experiencing a psychotic episode typically report auditory or visual hallucinations, hold paranoid or delusional beliefs, experience personality changes and exhibit disorganized thinking. Schizophrenia typically has its onset in late adolescence or early adulthood and presents as a constellation of positive (hallucination, delusions, disorganization of thought and bizarre behaviour), negative (loss of motivation, restricted range of emotional experience and expression and reduced hedonic capacity) and cognitive impairments, with extensive variation between individuals. Many patients with schizophrenia present comorbidities with depression and substance abuse contributing to the 10–15% lifetime incidence of suicide. No single symptom is unique to schizophrenia and/or is present in every case. Psychotic episodes, for example, are also not uncommon in cases of brain injury, learning disability, substance abuse and a range of metabolic disorders and may occur after chronic psychological stress and vary in duration between individuals. Psychosis is thus a descriptive term for a complex group of behaviours and experiences. Unfortunately, the current diagnosis and classification of schizophrenia is based solely on the physicians experience in interpreting clinical symptoms presented by the patient.

Schizophrenia and bipolar affective disorder are a major burden to affected individuals, their families and to society at large, affecting at least 2% of the population worldwide and costing hundreds of billions in health-care provision, treatments and lost earnings. Schizophrenia is found at

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similar prevalence in both sexes and throughout diverse cultures and geographic zones. The World Health Organization found schizophrenia to be the world's fourth leading cause of disability accounting for 1.1% of the total DALYs (Disability Adjusted Life Years) and 2.8% of YLDs (years of life lived with disability). It was estimated that the economic cost of schizophrenia exceeded US$ 19 billion in 1991, more than the total cost of all cancers in the United States. Epidemiological studies imply that effective treatments used early in the course of schizophrenia could improve prognosis and help reduce the costs associated with this illness. However, the current (non-standardized) mix of therapeutic interventions reduce the burden by only 13% (Saha *et al.*, 2005). Thus, any novel therapeutic interventions that reduce the overall cost of mental health provision, minimize adverse effects and/or offer a wider choice of effective drug classes (alone or combined with current antipsychotic drugs) will have a substantial impact on the care and life quality of the mentally ill, their relatives and society at large.

Biomarker discovery for schizophrenia has been carried out for more than 20 years (Prilipko, 1986). The field of biomarker discovery has frequently been regarded as unpromising, mostly due to the lack of useful markers that have so far emerged from this research. An important aim of biomarker discovery is the detection of molecular disease correlates that can be used as diagnostic tools. They should furthermore have predictive power and allow the identification of at-risk individuals. This is especially important, as early intervention is crucial for positive outcome of many diseases and increasing lines of evidence suggest that this also holds for psychiatric disorders (Holmes *et al.*, 2006; Killackey and Yung, 2007). In oncology, the usefulness of biomarkers for diagnostic purposes has long been demonstrated and routine assays are commercially available for these markers. For psychiatric disorders, this situation is very different and no molecular readouts have yet been generated that are of clinical use. The niacin test for diagnosis of schizophrenia was suggested in 1980 (Horrobin, 1980) following the observation of decreased skin flushing of schizophrenia patients after niacin application (Puri *et al.*, 2001, 2002; Tavares *et al.*, 2003; Bosveld-van Haandel *et al.*, 2006). The low sensitivity and heterogeneity of the response, however, prevented the niacin test from finding its way into clinical application. Although high sensitivity and specificity are crucial properties for a diagnostic application, only the knowledge of disease mechanisms will facilitate the discovery of novel, more efficient therapeutics and the development of animal models appropriate for schizophrenia. Therefore, the future success in advancing the clinical management of schizophrenia critically depends on a thorough understanding of pathognomonic alterations. The urgent need to understand disease mechanisms is underlined by the mentioned similarity of symptoms between different psychiatric disorders pointing to aetiologies that are possibly in part common to apparently diverse disorders.

For schizophrenia, arguably one of the most complex disorders to investigate, the yet missing success of biomarker discovery approaches can be ascribed to a combination of very high heterogeneity at the molecular level, difficulties to access appropriate samples, the absence of prominent and unique alterations, and an intricate interplay of genetic predisposition and environmental influences. Given these circumstances, large sample numbers are necessary to detect pathognomonic alterations associated with the disease state. Only recent technical developments facilitate these approaches and, in fact, several potential biomarkers have recently been reported for schizophrenia (Holmes *et al.*, 2006; Huang *et al.*, 2006). For psychiatric disorders, the magnitude of observed changes is usually small and mostly multiple molecules are affected. Therefore, it is likely that biomarkers that can ultimately assist the clinical management of schizophrenia will consist of multiple analytes. Here, we want to describe why the design of biomarker discovery studies can give very interesting insights into disease mechanisms and in turn substantially broaden the knowledge of the disease.

Multi-omics approaches for psychiatric disorders

Over the last few decades, a considerable number of hypotheses relating to factors involved in the aetiology of schizophrenia have emerged, several of which have been backed up by experimental evidence. These hypotheses have, however, not led to the discovery of novel diagnostic tools, mainly due to the occurrence of similar phenomena in other disorders or a high variability within the general population. Recent technological advances made it possible to leave hypothesis-based approaches of biomarker discovery behind in favour of non-hypothesis-driven profiling experiments. These data-driven approaches are likely to help untangle the complex phenomenon schizophrenia. In fact, non-hypothesis-driven approaches at the genetic level have been carried out in the form of linkage analyses for a long time (for example DeLisi *et al.*, 2002; Lewis *et al.*, 2003). These studies provided interesting insights into genes conferring an increased risk to schizophrenia. Despite the high heritability, single genes, however, only make a very small contribution to the overall risk of being affected by the disease, and the presence of even the most promising findings (such as, *neuregulin* 1) account for an increase in risk of only 1%. As the genetic contribution only accounts for a part of the risk of being affected by schizophrenia, the effect of environmental factors must not be underestimated. Interestingly, if an individual once develops a psychotic state, the probability for this person to relapse is greatly increased. Relapse rates can reach 80% in the second year after discharge from hospital (Csernansky and Schuchart, 2002). This implies that the complex interaction of genetic predisposition and environmental factors ultimately induces a molecular alteration that accounts for the highly increased risk of developing a chronic psychotic illness. As these alterations are dynamic by nature, metabolic and proteomic profiling methods seem especially suited for analysis. The complexity, probably inherent to the molecular changes in the psychotic state, additionally encourages non-hypothesis-driven approaches. For schizophrenia, these profiling experiments have to comprise large sample numbers of patients and very well-matched controls. Samples from other diseases presenting with similar symptoms should be included in the analysis to assess the disease-related specificity of potential biomarkers.

Profiling at the protein and metabolite level has not been possible on a large scale until recently, due to several technological limitations. The analysis of large sample numbers is a very critical element in studies aiming to define biomarkers for complex psychiatric disorders such as schizophrenia. At the protein level, MALDI (matrix-assisted laser desorption ionization) and SELDI (surface-enhanced laser desorption ionization)-based experiments allowed profiling of complex samples such as CSF. The identification of potential biomarkers is, however, not straightforward, if intact proteins are to be analysed. Liquid chromatography (LC)-based methods, in contrast, allow the direct analysis of complex, for example, digested protein samples enabling direct identification of analysed peptides. ICAT (isotope-coded affinity tags) and iTRAQ (isobaric tags for relative and absolute quantitation) are common methods allowing the quantification of these peptides. The limitation of these methods lies in the experimental variation introduced by the labelling as well as the limited sample number due to the availability of only few different labelling reagents. This problem can be overcome by the application of normalization techniques which aim to reduce batch effects (van der Greef *et al*., 2007). Label-free proteomics offers a more direct approach, where samples are analysed sequentially without previous labelling. Peptides are then directly compared between the samples. This approach is based on the observation that peptide intensities linearly correlate with the protein abundance in the samples (Bondarenko *et al*., 2002; Chelius and Bondarenko, 2002).

For profiling experiments in psychiatric disorders, the brain is the most interesting tissue to analyse. As the brain of living patients is obviously not clinically accessible, profiling experiments of brain tissue have to rely on post-mortem samples. Although these samples need to be analysed carefully to avoid post-mortem artefacts, they are a very valuable source for the generation of hypotheses with regard to the aetiology of a disorder. A parallel transcriptomics, proteomics and metabolomics study on human brain tissue in our laboratory has identified altered proteins, transcriptional and metabolite perturbations associated with glucoregulatory responses in schizophrenia (Prabakaran *et al*., 2004). Abnormal glucose profiles and a higher prevalence of type II diabetes (15.8% vs 2–3% in general population) in schizophrenia patients, coupled with reduced glycolysis and glycogenesis and enhanced glycogenolysis, were suggested to be connected to increased glucose demand and/or cellular hypoxia within the schizophrenia prefrontal cortex. This is an example how 'systems thinking' can be implemented in schizophrenia research and shows how multi-omics approaches based on different profiling platforms can give consistent experimental evidence for molecular alterations in psychiatric disorders.

For the development of diagnostics, these alterations have to be ultimately reflected in clinically accessible body fluids or tissues. CSF is especially interesting for psychiatric disorders. Owing to its proximity to the brain, CSF is likely to directly reflect pathological alterations in brain function. ^{1}H-NMR spectroscopy revealed elevated glucose levels in the CSF of first-onset drug-naive schizophrenic patients, but not in the serum samples of the same patients, suggesting that the glucoregulatory changes are brain-specific (Holmes *et al*., 2006). CSF has also been used for peptide/protein-profiling experiments and SELDI-TOF MS (SELDI time-of-flight mass spectrometry) revealed increased levels of a VGF peptide in first-onset schizophrenia patients (Huang *et al*., 2006). Interestingly, the discovered peptide featured differential processing, as a related peptide with the same sequence but three amino acids shorter, was not differentially expressed between schizophrenia patients and healthy volunteers. Furthermore, the use of two independent protein profiling techniques, SELDI-MS and two-dimensional-differential in gel electrophoresis (DIGE), revealed that apolipoprotein A1 (ApoA1) was significantly reduced in the CSF and peripheral tissues of schizophrenia patients (Huang *et al*., 2007) pointing to a role for Apolipoproteins in the CNS. Evidence of mitochondrial dysfunction and increased oxidative stress have been noted in Alzheimer's disease and schizophrenia, and changes in ApoA1, ApoE and other Apolipoproteins have been observed to change in Alzheimer's disease and various other forms of dementia (Reiss, 2005). Multiple studies have also reported altered levels of transthyretin in schizophrenia and other psychiatric disorders (Huang *et al*., 2006; Sullivan *et al*., 2006; Wan *et al*., 2006). These studies suggest a convergence of disease processes in several neuropsychiatric disorders that present with cognitive impairment.

Profiling approaches for the assessment of dynamic disease related alterations

For biomarker discovery in psychiatric disorder, careful experimental design is crucial to ensure clinical relevance of profiling results. In this context, the advantages of analysing drug-naive first-onset schizophrenia patients hold special importance. The molecular effects of antipsychotic treatment are not well understood. It is therefore difficult to exclude a contribution of drug treatment to the observed alterations in samples of drug-treated patients. The analysis of schizophrenia patients before and after drug treatment, along with information about clinical improvement, is, however, very valuable for the detection of potential biomarkers and promises to provide deeper insights into the disease mechanisms of schizophrenia. If biomarkers were to show an early response to drug treatment and were predictive of clinical outcome, the efficacy of medication could be quickly assessed and unsuccessful treatment could be reduced to a minimum. Methods aiming at the revelation of the genetic contribution to schizophrenia result in a static view of the disorders, marked by the presence or absence of certain risk conferring gene polymorphisms. The profiling of the proteome or metabolome, in contrast, results in quantitative measures of the abundance of molecules. This facilitates not only a more comprehensive understanding of complex systems in a disease like schizophrenia, but also allows tracing the disease-related alterations over the duration of the illness in a quantitative manner. This enables the assessment of treatment success and compliance at the molecular level and reveals how alterations intrinsic to the disease develop over time. In this context, the analysis of

prodromal patients is especially interesting, as the identification of factors ultimately leading to full-blown schizophrenia would be invaluable. The correlation of the detected alterations to the severity of the symptoms promises to give particularly valuable insights into factors most important for the development of mental disorder. The presence of the diverse clinical manifestations mentioned earlier led to the hypothesis that the disorder comprises various subtypes with different aetiologies. Quantitative measures of molecules might facilitate the subclassification of schizophrenia, which could ultimately result in a biochemical redefinition of the disorder.

Various animal models exist for schizophrenia, the most common of which is phencyclidine treatment of rats. Besides hyperdopaminergia and hypoglutaminergia, treatment with this NMDA-receptor antagonist induces behavioural changes similar to positive and negative symptoms of Schizophrenia (Javitt and Zukin, 1991; Olney *et al.*, 1999). Besides the great similarity of symptoms, the validity of these animal models remains to be established. Profiling studies can make a great contribution in this field by assessing the similarity of molecular alterations on a system level if changes in animal models reflect changes observed in patients developing psychosis. These models also allow the analysis of antipsychotic treatment effects or other confounding factors such as cannabis consumption in a controlled experimental setup. The knowledge of the molecular effects of these factors will be a great advantage for the discovery of alterations inherent to the disease as datasets can be efficiently curated.

Multi-omics approaches based on the analysis of different body fluids and tissues with various profiling platforms promise to provide deeper insights into complex psychiatric disorders. Especially for schizophrenia, which is marked by high heterogeneity and biological variability, the experimental setup of profiling approaches seems suited for the discovery of disease mechanisms. Profiling platforms can capture dynamic pathognomonic alterations, their response to antipsychotic treatment and the contribution of environmental factors to the onset. Therefore, biomarker discovery experiments based on profiling approaches facilitated by recent technical development are likely to make a great contribution to uncovering disease mechanisms in complex psychiatric disorders.

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Conflict of interest

The authors state no conflict of interest.

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