Advances in Schizophrenia Research

Chapter 4

Blood Based Genetic Biomarkers of Schizophrenia

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Abstract

Schizophrenia (SZ) is a disabling, chronic, and severe brain disorder with collective symptoms such as delusions, hallucinations, disorganized speech or behavior, impaired cognitive ability etc. Although multiple factors are responsible for the disease phenotypes, the precise cause of disease pathogenesis is debatable. But, genetic susceptibility and environmental influences play a vital role in pathophysiology. Additionally, derangements to the central nervous system also influence the gene expression and metabolism in the peripheral blood via several factors viz. neurotransmitters, hormones, cytokines, immune-related alterations. Therefore, identification of blood-based biomarkers is reliable and feasible option to examine heterogeneous disease-causing factors such as nucleic acids, metabolites, epigenetic features, gene expressions etc.; however, examining brain related markers are not yet well-established. Substantial research work suggests deregulation of several genes in the disease condition and reported to be increased risk for schizophrenia appeared to be affecting memory-related signaling cascades, RNA processing, DNA replication, signal transduction, cytokine signaling etc. It is interesting

to note that more than 70% identified schizophrenic biomarkers were found to be associated with the inflammatory response of the cell and the level of inflammatory molecules were reported to be varying with the severity, duration, and therapy given to patients. Detection of genetic-epigenetic factors from blood samples in SZ patients have shown a great clinical importance in order to find candidate diagnostic and/or prognostic biomarkers as well as target molecules which can be crucial for therapeutic purposes.

Keywords: Schizophrenia; Gene expression; Blood-based biomarkers; Molecular diagnosis.

1. Introduction

Schizophrenia (SZ) is a common disorder with a lifetime prevalence of ~1% [1]. It affects one's thought and sense of self. The mood disturbances are not primary features which distinguish SZ from other mood disorders. Similarly, there may be mild impairment of cognitive function, and it is distinguished from the dementias in which disturbed cognitive function is considered primary. There is no characteristic pathology, such as neurofibrillary tangles in Alzheimer disease. It is highly heritable but the genetics of SZ is complex and the molecular mechanisms behind pathophysiology of SZ are still ambiguous and is a matter of intense research. This situation impedes progress in diagnosing the patients at early stages, risk assessment and the development of rationally selected therapeutics to alter disease progression and clinical trajectory.

Characterizing molecular mechanisms correlate of SZ is of great potential interest and value. In the past 15 years, the transcriptome (all the messenger RNA molecules expressed from all coding genes of an organism) has received major attention in SZ research, particularly in the effort to identify biomarkers of normal functioning or illness. The study of the whole transcriptome has been reached to new heights by microarray and sequencing (Next generation sequencing; NGS). Both technologies provide several advantages such as efficient and unbiased identification, multiplexing (transcriptome of many samples can be identified/quantified in single experiment), differential expression (between two conditions viz. patients versus healthy controls, drug responders versus non-responders etc.) and post-experiment analysis like, measurement of large numbers of biological features allows for the assessment of network functions, altered molecular pathways identification and to generate new hypotheses about the biological phenomenon.

2. Potential of Blood Testing in Schizophrenia

Being a complex and heterogeneous disorder, findings from the studies in brain tissue have not yet been translated into biomarkers that are practical in clinical use because brain biopsies are not acceptable and neuroimaging techniques are expensive and the results are inconclusive. Therefore, search for blood-based biomarkers of SZ has increased in recent years

as a valid alternative.

Blood is a non-invasive source of investigating biomolecules associated with the pathophysiology of the disease. It is a connective medium by which secretary and excretory products are transported to their target regions and also provide as a feasible option where a distinct pattern of metabolites, nucleic acids, epigenetic factors can easily be examined related to several disease pathophysiology. Diagnosis in the early stages of disease is perhaps the most critical time window **(Figure 1)**. It is well established now that CNS influences the gene expression and metabolism in the peripheral blood *via* several ways viz. cytokines, neurotransmitters, or hormones, while immune-related alterations in the CNS may, in turn, originate from peripheral blood. Therefore, blood in SZ patients has been extensively investigated to identify the candidate's diagnostic, prognostic biomarkers and also for molecules which can be targeted for therapeutic purposes.

Schwarz and colleagues described a 51-plex assay that was capable of discriminating schizophrenia patients from healthy controls with good performance [2]. The most robust analytes included in this assay were related to immune or inflammatory functions although other pathways were also represented such as hormonal signalling, response to stress, growth factor signalling, and metabolism. However, future biomarker-based studies are necessary to develop more sensitive and specific new blood-based biomarker assays which can predict disease development, potentially in help-seeker individuals meeting the ultra-high-risk criteria. In turn, early diagnosis and intervention will enable a better therapeutic effect and prevent devastating consequences of the disease.

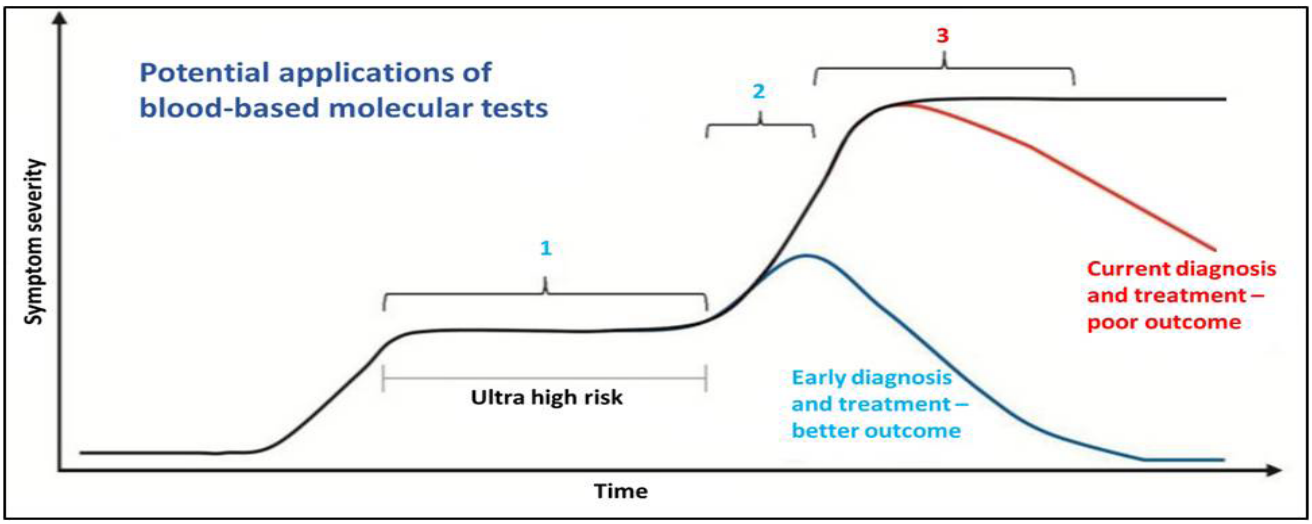

Figure 1:-Diagram illustrating disease progression in schizophrenia and potential applications of blood-based-molecular tests. (1) Early diagnosis – estimating the risk of developing schizophrenia. (2) Treatment response prediction – accurately identifying subjects who will benefit from antipsychotic treatment. (3) Patient monitoring/risk for side effects and relapse – monitoring biomarker concentration changes during and after treatment.(Acknowledgements-Chan and Colleagues [3].

3. Differential Gene Expression in Blood

Significantly reduced blood BDNF (brain derived neurotrophic factor) levels [4,5] including hypermethylation of its promoter [6] has been positively correlated with cognitive functions like semantic generation tasks [7] and auditory processing after computerized cognitive training [8] among SZ patients. However, reduced levels of BDNF were also found in the plasma of patients with bipolar disorder [9] and major depressive disorder [10]. Even, reduced levels of BDNF in SZ patients requires more investigation as there are reports showing conflicting results on the same [5,11,12]. Recently, a significantly high level of BDNF have been reported in the serum of SZ patients (n=56) as compared to healthy controls [13]. Hence, specificity of these biomolecules is compromised and therefore, more longitudinal studies are required to determine whether altered blood BDNF levels is a characteristic biomarker for the disorder or has some prognostic value. Recently, in old patients of SZ, a significant positive association has been reported between higher serum levels of BDNF and greater severity for the negative symptoms like passive, apathetic, social withdrawal, and emotional withdrawal reflecting compensatory neuronal mechanisms resulting from neurodevelopmental dysfunction [14].

TCF4, a transcription factor, was one of the first genes to reach genome-wide significance in large-scale genetic association studies of SZ [15] and different single-nucleotide polymorphisms (SNPs) have been associated with the disorder in independent studies [16,17]. It is widely expressed in the brain and plays a pivotal role in neurodevelopment. Altered levels of TCF4 have been associated with cognitive impairments and deficits in pre-pulse inhibition in mice over-expressing TCF4 in the forebrain [18]. It has been also shown that peripheral expression of TCF4 is significantly correlated with grey matter thickness in the prefrontal cortex [19]. Recently, significant decreased mRNA levels of TCF4 gene was found in peripheral blood of SZ patients (n=70). Altered TCF4 levels also showed significant negative correlation with WCST and PANSS scores in patients [20].

An integrated study using microarray and neural networking technologies showed the process of cell adhesion as a significantly over-represented gene ontology including a gene-set of CINP, TDRD9, DAOA, PGRMC1, NAF1, LIPH, MAP1D and INSL3 genes. The study used whole blood of 52 antipsychotic-naive schizophrenia patients and 49 healthy controls. The microarray found 792 differentially expressed genes while neural network approach identified significant involvement of cell adhesion process in SZ pathology [21]. Altered levels of adhesion molecules, vascular endothelial growth factor (VEGF), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in blood plasma of 99 patients and 99 healthy controls were reported [22]. Patients with higher these three molecules were found to have an earlier age of disease onset, higher systolic and diastolic blood pressure, lower high-density lipoprotein cholesterol, higher insulin resistance, lower

levels of mental well-being, and higher Framingham Coronary Heart Disease Risk scores.

The exome sequencing of SZ cases (n=2536) versus control (n=2543) demonstrated a polygenic burden primarily arising from rare (less than 1 in 10,000), disruptive mutations distributed across many genes [23]. Involvement of voltage-gated calcium ion channel and the signalling complexes formed by the scaffold protein ARC of the postsynaptic density, NMDAR network genes were primarily revealed by gene-set enrichment analysis. Calcium signalling is involved in many cell functions including regulating gene expression [24] and is critical for modulating synaptic plasticity [23,25].

Another exome sequencing of de novo mutations in SZ, using genomic DNA from 623 SZ trios demonstrated that small de novo mutations, affecting 1 or a few nucleotides, are overrepresented among glutamatergic postsynaptic proteins comprising an activity-regulated cytoskeleton-associated protein and NMDA receptor complexes [26]. The identified mutations were linked to proteins namely, proteins regulating actin filament dynamics and those whose mRNAs are targets of fragile X mental retardation protein etc. which interact with these complexes to modulate synaptic strength. The mutated genes in SZ were similar to those found in autism and intellectual disability, as do mutation-enriched synaptic pathways. Fromer and his colleagues aligned their findings with a parallel case-control study, which demonstrated reproducible insights into etiologic mechanisms for SZ and revealed pathophysiology shared with other neurodevelopmental disorders.

The RNA-sequencing from the whole blood of 36 drug-naive schizophrenia patients and 40 healthy controls revealed 200 differentially expressed genes. Some of the identified genes overlapped with genome-wide association studies of the disorder (including CSMD1, EHF, and RFX2) and others had been previously described in schizophrenia literature (GRIK3, LPL, S100B, SNCA, SYN2, TUBB2A and SELENBP1). Gene ontology enrichment in the differentially expressed genes suggested a key role for wounding, and acute inflammatory and innate immune response [27]. The involvement of immune-related genes was also shown by Gardiner and colleagues by sequencing RNA of PBMCs (peripheral blood mononuclear cells) from 114 cases of schizophrenia or schizoaffective disorder and 80 healthy controls. They showed significant and validated expression changes of EIF2C2, EVL, DEFA4, S100A12, PI3, and MEF2D in the cohort under investigation which provided a strong indication of a role for immune gene involvement in SZ [28].

Genome-wide RNA expression profiling of blood samples from 121 individuals diagnosed with schizophrenia (with 92 medicated and 29 anti-psychotic treatment free) and 118 healthy controls used weighted gene co-expression network analysis, in which ‘modules’ of genes are identified that co-vary in expression between cases and controls. Using arrays, De Jong and co-workers showed a schizophrenia-associated cluster of co-expressed genes

that were independent of medication-effects and the same was enriched with brain-expressed genes and regulated by MHC-complex [29]. The identified genes ABCF1, SLC2A6, SDHA, DHRS1, Sep-06, CNDP2, SIGIRR, FBXL5, and DHX58. Another cluster of genes included CCL5, PRKCQ, PTAFR, AKR1B1, CD247, IL10RA and KHSRP which was grouped into the Neurological Disease category and has well associated with SZ.

In a recent report, Moretti and colleagues have reported increased expression of CNR1 and UFD1L genes in SZ and treatment-resistant patients (failure to respond to two previous antipsychotic trials) when compared to controls [30]. The upregulated levels show their potential association with the release of neurotransmitters, which can influence neuronal plasticity, or with a stress response-activating protein degradation. Another set of genes, DICER1 and AKT1 were found to be upregulated across the groups. These are important genes of several heterogeneous pathways, such as cell signalling and miRNA processing. This all demonstrates that there is yet veiled genes or gene sets which either directly affect SZ pathogenesis or can be established as biomarkers.

4. Inflammation and Immune Function

More than 70% of potential biomarkers for the SZ are involved in the inflammatory response [31]. The levels of immunological processes or inflammatory molecules vary with the severity, duration, and therapy given to patients. Upregulated levels of pro-inflammatory cytokines (*i.e.*, IL-6, IL-2, TNF, IL-1β and IL-8) or some of their receptors [*i.e.*, IL-2 receptor-α (IL-2Rα), IL-1 receptor antagonist have widely been reported in the serum of SZ patients [31, 32]. The expression levels of *TNF*-α and *IL-1*β genes were found upregulated and interferon-γ (IFN-γ) were reduced in blood cells (PBMCs) of SZ patients as compared to matched controls [33].

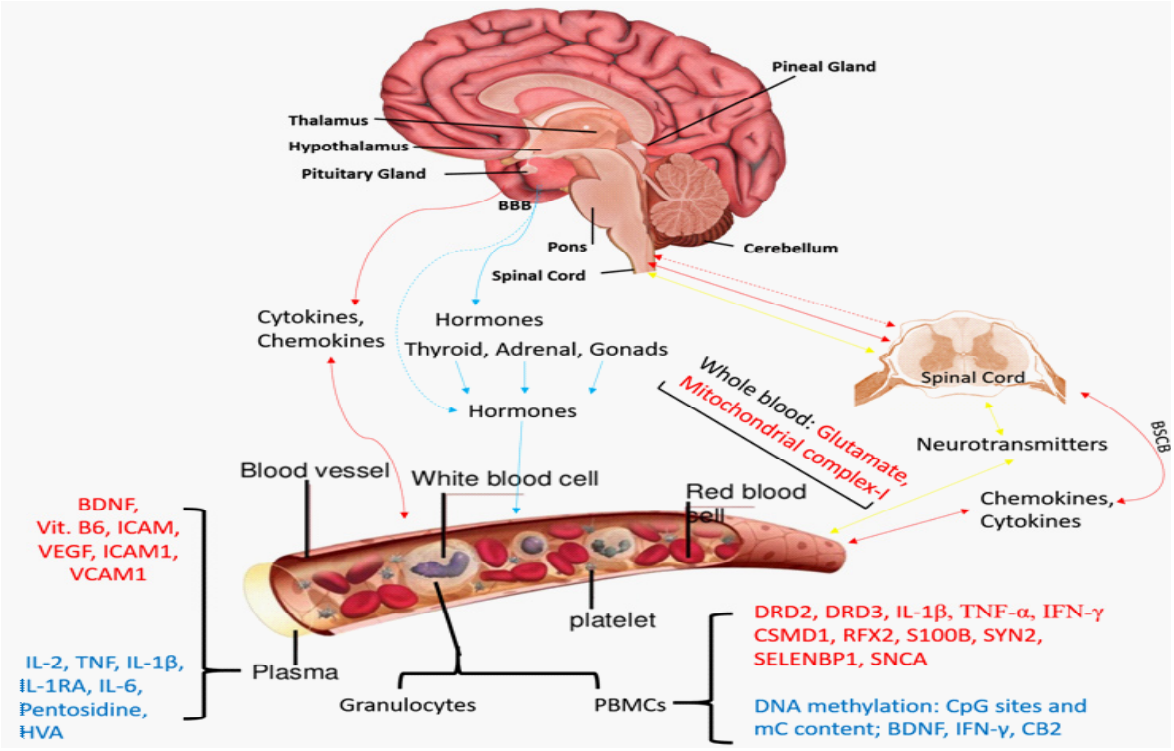

Figure 2: - A schematic representation of central nervous system-peripheral blood tissue interactions. CNS stress may influence gene expression, DNA methylation, and cell metabolism in the peripheral blood via cytokines, neurotransmitters, or hormones with different transportation methods. Cytokines or chemokines can transport across the BBB (red lines) or BSCB (orange line) either from CNS to peripheral blood tissue or vice versa. The hormones are exerted by CNS and transported across the BBB via blood system to the target tissue (blue lines) and in turn, regulate CNS through negative feedback (blue dashed lines). Another connection is the stimulated (yellow line) or negative feedback inhibition (yellow dashed line) via spinal cord via the parasympathetic or sympathetic nervous system. It is noted that there are several blood cell types with their own features in the peripheral blood vessel. [Modified from 34].

Here also, it is important to note that some of the pro-inflammatory cytokines such as IL-6, TNF, and IL-1 have also been reported high in the serum of patients with bipolar disorder. High levels of intracellular molecules involved in pro-inflammatory pathways in PBMC (*i.e.*, nuclear factor κB, inhibitory complex IκB, inducible isoforms of nitric oxide synthase, cyclooxygenase) alongside significant decreases in some anti-inflammatory proteins (*i.e.*, prostaglandin 15-dexoy-PGJ_2 and peroxisome proliferator activated receptor-γ). The results indicate that the peripheral markers may change at different disease stages, but together suggest that the peripheral immune system is over-activated in both individuals undergoing their first episode of psychosis and people with SZ.

It is clearly evident that many immunological molecules are common among patients with SZ and bipolar disorder which reflect commonalities between these two psychiatric diseases. Sensitivity and dynamic nature of the immune system add up diversity in expression levels of immunological processes and molecules. "Therefore, immunological profiles between SZ patients differ, as reported by various research groups [31,35,36,37,38]. Moreover, the antipsychotic and other drugs change their levels adding up the variations. More research is needed to determine the immunological pattern of SZ patients [34]. High levels of macrophage migration inhibitory factor (MIF), a pleiotropic cytokine was recently reported high in the serum of SZ (51) patients [39]. The MIF is involved in the regulation of innate and adaptive immunity and plays important role in inflammation and neurogenesis. Proteomic analysis of SZ patients has earlier showed MIF an important biomarker [40,41].

Schizophrenia-associated changes in immunoglobulin levels in both plasma and CSF support an immunological role in the pathogenesis of the disease [42]. Schizophrenia Working Group of the Psychiatric Genomics Consortium identified 128 independent gene associations using a multi-stage schizophrenia genome-wide association study of up to 36,989 cases and 113,075 controls [43]. Out of these 128 gene associations, Whelan and colleagues demonstrated different levels of plasma IgG antibodies against protein-derived fragments encoded by 18 genes. The SZ patients (n=356) were found to carry increased levels of circulating IgG antibodies for DPYD, MAD1L1, ZNF804A, DRD2, TRANK1, and MMP16 derived antigens and low levels IgG antibodies against TSNARE1, TCF4, and VRK2 derived antigens [44] compared to matched healthy controls. The anti-TRANK1 IgG levels revealed highest sensitivity (20.7%) against specificity (95.2%) among all 18 tests and are more likely to serve as a biomarker for SZ.

5. Summary

The pathophysiological mechanism of SZ remains elusive with contributing risk factors such as heterogeneous genetic and varied neurological phenotypes. With the help of high throughput technologies, multiple risk genes associated with the pathogenesis of SZ were clarified. Although analysis of both blood and brain tissues of SZ patients were demonstrated, blood-based markers have great clinical applications as it is a connective medium by which secretary and excretory products are transported to their target regions and helpful in understanding disease complexity at various levels. Majority of markers were found to be associated with immune system leading to SZ pathogenesis. Moreover, research studies also suggest their implications for other psychiatric disorders too. Deregulation of several genes have been reported for increased risk for schizophrenia appeared to be affecting memory-related signaling cascades, RNA processing, DNA replication, signal transduction, cytokine signaling etc. Not a single molecule, at present, can be called as perfect biomarker for SZ. The genome-wide profiling is required at a very large scale at different stages of disease to find non-overlapping biomarkers which in turn will also provide new insight into the disease pathogenesis and help scientists and clinicians to develop better diagnostic and treatment methods for SZ patients.

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