

Consensus on potential biomarkers developed for use in clinical tests for schizophrenia

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ABSTRACT

Background Schizophrenia is a serious mental illness affecting approximately 20 million individuals globally. Both genetic and environmental factors contribute to the illness. If left undiagnosed and untreated, schizophrenia results in impaired social function, repeated hospital admissions, reduced quality of life and decreased life expectancy. Clinical diagnosis largely relies on subjective evidence, including self-reported experiences, and reported behavioural abnormalities followed by psychiatric evaluation. In addition, psychoses may occur along with other conditions, and the symptoms are often episodic and transient, posing a significant challenge to the precision of diagnosis. Therefore, objective, specific tests using biomarkers are urgently needed for differential diagnosis of schizophrenia in clinical practice.

Aims We aimed to provide evidence-based and consensus-based recommendations, with a summary of laboratory measurements that could potentially be used as biomarkers for schizophrenia, and to discuss directions for future research.

Methods We searched publications within the last 10 years with the following keywords: ‘schizophrenia’, ‘gene’, ‘inflammation’, ‘neurotransmitter’, ‘protein marker’, ‘gut microbiota’, ‘pharmacogenomics’ and ‘biomarker’. A draft of the consensus was discussed and agreed on by all authors at a round table session.

Results We summarised the characteristics of candidate diagnostic markers for schizophrenia, including genetic, inflammatory, neurotransmitter, peripheral protein, pharmacogenomic and gut microbiota markers. We also proposed a novel laboratory process for diagnosing schizophrenia in clinical practice based on the evidence summarised in this paper.

Conclusions Further efforts are needed to identify schizophrenia-specific genetic and epigenetic markers for precise diagnosis, differential diagnosis and ethnicity-specific markers for the Chinese population. The development of novel laboratory techniques is making it possible to use these biomarkers clinically to diagnose disease.

INTRODUCTION

Schizophrenia is a chronic disorder characterised by continuous or relapsing episodes of psychosis, with hallucinations, delusions

Key messages

What is already known on this topic

► Schizophrenia is a complex mental illness. The precise and early diagnosis of schizophrenia is challenging.

What this study adds

► This study includes a comprehensive introduction of the characteristics of candidate diagnostic markers for schizophrenia and a novel laboratory process for diagnosing schizophrenia in clinical practice.

How this study might affect research, practice or policy

► This study provides a first-hand laboratory testing basis for the clinical diagnosis of schizophrenia.

and disorganised thinking as major manifestations.¹ Schizophrenia has a lifetime prevalence of approximately 0.3%–0.7%² and mainly affects teenagers and young adults and more males than females (the male-to-female ratio is approximately 1.4:1).³ Other risk factors include advanced paternal age, perinatal events, influenza or other infections in the second trimester, birth in spring and drug use.^{4–6} The clinical diagnosis of schizophrenia is based on the criteria in the Diagnostic and Statistical Manual of Mental Disorders edition 5 or the International Statistical Classification of Diseases and Related Health Problems edition 11. For schizophrenia, the presence or absence of specific first-rank positive symptoms (including auditory hallucinations, thought withdrawal, insertion or interruption; thought broadcasting; somatic hallucination; delusional perception; feelings or actions of being controlled by external agents) may be particularly helpful for clinical diagnosis.⁷ However, negative symptoms (including affective flattening, avolition, alogia and anhedonia) should not be neglected. Although

they are less prominent during the acute phase, they reflect diminished emotional expression and reduced motivation and behavioural activities.⁸

The precise and early diagnosis of schizophrenia is challenging because the pathological mechanisms of the disease are not fully understood. The current diagnostic criteria are based on subjective evidence; however, psychotic episodes are transient and difficult to detect, especially during the early stage of the disease. Therefore, objective and specific biomarkers by laboratory testing are urgently needed in the clinical diagnosis of schizophrenia. In general, these biomarkers are classified into three categories: (i) persistent markers such as genetic markers, (ii) episodic and symptom-related markers such as inflammatory markers and (iii) markers as indicators of treatment responsiveness, such as pharmacogenomic markers. The major goals of testing these biomarkers are to (i) identify the risk of schizophrenia to benefit early diagnosis and intervention, (ii) assist in diagnosis and differential diagnosis, (iii) evaluate disease severity and progression, (iv) predict suicide risk and (v) assist in individualised treatment strategies by testing markers related to drug efficacy and side effects.

The objectives of this study were to (i) discuss the existing evidence for laboratory measurements as potential diagnostic tools, (ii) propose a novel laboratory process for the recognition of schizophrenia for use in clinical practice and (iii) suggest the design of laboratory and clinical studies by outlining the further investigations required. We hoped to comprehensively summarise the currently available approaches and trigger more clinical research to improve diagnostic tools and technologies.

METHODS

We searched PubMed, Embase and the Cochrane Library for publications within the last 10 years with the following keywords: 'schizophrenia', 'gene', 'inflammation', 'neurotransmitter', 'protein marker', 'gut microbiota', 'pharmacogenomics' and 'biomarker'. An initial draft for circulation was developed by the Shanghai Mental Health Center and subsequently discussed at a round table session at the 2021 Annual Meeting of Laboratory Medicine of the Chinese Medical Doctor Association held between 12 and 14 May 2021.

Existing evidence for laboratory measurements as potential diagnostic tools

Genetic markers

Schizophrenia has strong heritability, estimated to be between 70% and 80%.⁹ Having a first-degree relative with the disease has been considered the greatest risk factor for developing schizophrenia, and over 40% of identical twins of those with schizophrenia are also affected.¹⁰ Previous studies have shown that a higher genetic risk of schizophrenia is associated with worse cognitive performance,¹¹ and early-onset cases appear to be associated with a higher rate of large cytogenetic abnormalities and

rare structural variants than those reported in late-onset cases.¹²

Copy number variants (CNVs) have been considered to induce greater risk, and individuals with schizophrenia have an increased genome-wide CNV burden,¹³ although such changes have been found in only 2%–3% of patients with schizophrenia.¹⁴ One of the most recognised CNVs is a deletion at 22q11.2, which has been estimated to have a prevalence of 1%–2% in schizophrenia cases and is also seen in early-onset cases (22q11.2 deletion syndrome is also known as the velocardiofacial syndrome).^{15–17} In addition, it has been estimated that up to 25% of adults with the deletion have schizophrenia,¹⁸ and the deletion induces a 30% lifetime risk for schizophrenia.¹⁹ In conclusion, the deletion at 22q11.2 may play a crucial role in disease development and progression. Other CNVs, such as deletions at 17q12, duplications at 16p11.2 and deletions at 15q11.2, have also been reported to increase the risk of developing schizophrenia and are often comorbid with autism and intellectual disabilities.^{17 20} In terms of ethnic factors, genetic studies comparing large East Asian cohorts with European cohorts revealed similarities in the effects of common genetic variants on the risk of schizophrenia.²¹

Although individual loci are considered to have relatively minor effects, an association between these loci and schizophrenia symptoms has been established, and schizophrenia is considered highly polygenic. Large cohort genome-wide association studies (GWAS) have identified >100 loci that are associated with schizophrenia, including loci in major histocompatibility complex (MHC) regions, microRNA-137 (*MIR137*), zinc-finger protein 804A (*ZNF804A*), neurogranin (*NRGN*) and transcription factor 4 (*TCF4*)^{22–24}; among these associations are enriched within genes expressed in tissues that play critical roles in immunity, particularly B-lymphocyte lineages involved in acquired immunity, providing evidence for the hypothesised link between the immune system and schizophrenia.²² The identified loci overlap and vary in different ethnicities. For instance, studies on cohorts with European ancestry or a combination of European and African ancestry revealed schizophrenia-related single-nucleotide polymorphisms (SNPs) for *TRIM26*, *CCDC68*, *TCG4*, *MIR137*, *DPYD-MIR137*, *PCGEM1*, *CSMD1*, *MMP16*, *CNNM2*, *NT5C2*, *STT3A*, *AMBRA1*, *DGKZ*, *CHRM4*, *MDK*, *ITIH3*, *ITIH3-ITIH4*, *AS3MT*, calcium channel subunits *CACNA1C* and *CACNB2*, human leucocyte antigen (*HLA*)-C*01:02, *HLA-DRB9* and other multiple loci in the MHC region; *SDCCAG8*, *C10orf26*, *NRGN*, *ZNF804A*, ankyrin-3 (*ANK3*), hepatocyte nuclear factor 4γ (*HNF4G*), NADH dehydrogenase (ubiquinone) iron-sulfur protein 4 (*NDUFS4*), histone deacetylase 9 (*HDAC9*) and various other loci.^{11 13 24–32} In addition to some of the previously identified loci, one study in patients with Chinese ancestry revealed four novel loci at 3p21.31, 6q21, 6q27 and 7q31.1.³³ However, a study in a Japanese cohort did not show any locus of genome-wide significance but supported a polygenic risk for schizophrenia.³⁴

Some of these loci have been associated with genes that contribute to disease manifestation. For instance, rs830786 within *HNF4G* has been associated with attention/vigilance, and rs67017972 near *NDUFS4* has been associated with verbal memory, whereas rs76872642 within *HDAC9* has been associated with reasoning and problem-solving.¹¹ In addition, *ANK3* variants have been associated with lower cognitive performance, with the risk allele rs1938526 particularly associated with the domains of verbal memory, working memory and attention,³⁵ whereas *NRGN* variants have been associated with episodic and working memory.³⁶ One study suggested the involvement of rs6994992 within neuregulin 1 in the susceptibility to developing cognitive deficits in patients at the first stage.³⁷ Variants of *GRM5* (coding for metabotropic glutamate receptor 5 (mGluR5)) have been shown to be associated with cognitive impairment.³⁸ Additionally, SNPs in dystrobrevin-binding protein 1 have been associated with a variety of cognitive domains in schizophrenia, including attention, vigilance, memory and processing speed.^{39,40} Furthermore, genes related to neurotransmitters have also been linked to cognitive impairment in schizophrenia. For instance, the 5-hydroxytryptamine 2A (5-HT_{2A}) receptor gene polymorphism is associated with sustained attentional impairment in patients with early-onset schizophrenia.⁴¹ Moreover, the dopamine β-hydroxylase (DβH) polymorphism may be linked to DβH activity in the Chinese population and may influence some aspects of cognitive function in schizophrenia.⁴²

Some of these loci have been associated with changes in brain structures. Genetic variations of *CACNA1C*, *NRGN*, *TCF4* and *ZNF804A* have been associated with grey matter volume, and variations in *ANK3* and *ZNF804A* have been associated with white matter integrity.³⁶ Variations in *ANK3* have been linked to the brain structure, and *NRGN* variations have been linked to brain volume.³⁶ Specifically, the risk allele of *ANK3* (rs1938526) is associated with widespread cortical thinning in patients with first-episode psychosis,³⁵ and *GRM5* variants are associated with a reduction in right hippocampal volume in schizophrenia without affecting mGluR5 protein expression.³⁸

Other loci have also been associated with potential mechanisms. For instance, *ANK3*, *CACNA1C*, diacylglycerol kinase eta, *NRGN* and *ZNF804A* have all been associated with regional activation during executive tasks, and *CACNA1C* and *ZNF804A* are associated with functional connectivity during executive tasks.³⁶ *MIR137* plays an essential role in synaptic plasticity,⁴³ and some of its targets include GWAS-identified schizophrenia genes.^{44,45} The MHC region, which encodes genes critical to immunity, is involved in susceptibility to schizophrenia, and risk associated with *NRGN* and *TCF4* points to perturbation of pathways involved in brain development, memory and cognition.^{30,46} The catechol-O-methyltransferase gene Val allele slightly increases the risk of schizophrenia with a potential mechanism involving increasing prefrontal dopamine catabolism, thus impairing prefrontal cognition and physiology.⁴⁷ Furthermore, these genomic loci

may also be present in specific neuronal cell types. For instance, single-cell RNA sequencing has revealed that common variants are consistently present in pyramidal neurons, medium spiny neurons and specific interneurons, but to a much lesser extent in glial cells, indicating specific roles they may play in the disease mechanisms.⁴⁸

Interestingly, schizophrenia has been shown to share some common risk variants with other psychiatric diseases, with the highest SNP co-heritability between schizophrenia and bipolar disorder, followed by schizophrenia and major depressive disorder, suggesting potential common pathways among different psychiatric diseases.⁴⁹

Environmental factors, which have been linked to changes in gene expression via epigenetic modulation, have also been considered risk factors for developing schizophrenia. Previous analyses of gene methylation in schizophrenia have mainly focused on neurotransmitters, such as γ-aminobutyric acid (GABA), glutamate, serotonin and dopamine, and various candidate genes have been identified by analysis of brain tissues, whole blood and saliva.⁵⁰ DNA methylation of the reelin and glutamic acid decarboxylase genes (*RELN* and *GADI*, respectively), which are involved in the GABAergic pathway, has been identified in the brains of patients with schizophrenia,^{51,52} whereas DNA methylation of the serotonin receptor type-1 gene (*HTR1A*) is increased in the peripheral blood of patients with schizophrenia compared with controls.⁵³ Notably, one study has revealed that the most significant differentially methylated positions in the Chinese cohort are the *C17orf63*, THAP domain-containing protein 1 and potassium voltage-gated channel subfamily KQT member 4 genes.⁵⁴

In addition to DNA methylation, changes in microRNA (miRNA) expression are observed in postmortem brains of patients with schizophrenia.⁵⁵ A previous study identified an increase in miR-328, miR-17-5p, miR-134, miR-652, miR-382 and miR-107 in the brains of patients with schizophrenia.⁵⁶ A meta-analysis of studies on peripheral blood mononuclear cells revealed decent sensitivities and specificities of miR-181b-5p, miR-21-5p, miR-195-5p, miR-137, miR-346 and miR-341-5p for the diagnosis of schizophrenia.⁵⁷

Inflammatory markers

Inflammation is considered to play a crucial role in the pathogenesis of schizophrenia. A postmortem study revealed decreased interleukin (IL)-1α and interferon (IFN)-γ-inducible protein 10 expression as well as increased IFN-α expression in the superior temporal gyrus of patients with schizophrenia,⁵⁸ suggesting a direct association of inflammatory markers with the disease. Since then, a variety of inflammatory markers have been assessed in different types of biospecimens, including peripheral blood and cerebrospinal fluid (CSF).

C reactive protein (CRP) has been shown to increase in schizophrenia and to be associated with disease severity.^{59,60} A meta-analysis revealed that CRP is

significantly associated with cognitive function in schizophrenia.⁶¹ In addition, serum levels of IL-1RA, IL-6, IL-7, IL-8, IL-9, IL-10, IL-13, IFN- γ , eotaxin-1, granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein 1 (MCP-1, also known as CCL2), platelet-derived growth factor subunit B, monocyte inflammatory proteins (MIP-1 α and MIP-1 β), vascular endothelial growth factor A and regulated on activation, normal T cell expressed and secreted (also known as CCL5) have all been shown to increase in patients with multi-episode schizophrenia.⁶² In addition, a cytokine imbalance of T helper types 1 and 2 has also been reported to be present in peripheral blood as well as in CSF.⁶³ Furthermore, CSF levels of IL-6 and IL-8 have been shown to be elevated in schizophrenia.⁶⁴ These inflammatory markers may also be able to predict the prognosis of schizophrenia, as higher baseline blood IL-6 is correlated with a greater reduction in the Positive and Negative Syndrome Scale (PANSS) total and general subscale scores at 3 and 6 months and PANSS negative subscale scores at 3 months.⁶⁵ Therefore, changes in these inflammatory markers may potentially serve to predict disease progression and manifestation.

In addition, changes in these inflammatory markers have been shown to be directly associated with the disease mechanisms. For instance, it has been hypothesised that inflammation interferes with cellular pathways, inducing the metabolism of tryptophan to kynurenine acid in schizophrenia. This is supported by evidence that the plasma level of kynurenine is positively correlated with IL-1 β and PANSS,⁶⁶ and the elevation of IL-6 and tumour necrosis factor- α is associated with the kynurenine pathway.⁶⁷

Importantly, these inflammatory markers may predict disease progression and drug efficiency. A meta-analysis has shown that the elevation of IL-1 β , IL-6 and transforming growth factor- β in acutely relapsed patients and first-episode psychosis could be normalised by antipsychotic treatment, whereas the elevation of IL-12, (IFN- γ), TNF- α and soluble IL-2 receptor (sIL-2R) in acute exacerbations is not reversible following antipsychotic treatment.⁶⁸ Therefore, inflammatory markers serve as important biomarkers for the prognosis of disease progression and treatment response in schizophrenia.

Apart from changes in these inflammatory markers, autoimmune dysfunction and maternal/perinatal infections have also been considered risk factors for schizophrenia.⁶⁹ Patients with schizophrenia who have a first-degree relative with schizophrenia are more likely to have a parent or sibling with an autoimmune disease,⁷⁰ and a history of autoimmune diseases increases the risk of developing schizophrenia by 29%.⁷¹ Increased risks of systemic lupus erythematosus, Graves' disease, coeliac disease and various other autoimmune diseases have been reported in patients with schizophrenia^{72 73}; however, a reverse association with rheumatoid arthritis is also observed.^{72 74}

Neurotransmitter markers

Three main theories have been put forth for the pathophysiology of psychosis: serotonin theory, dopamine theory and glutamate theory (N-methyl-D-aspartate hypoactivity).⁷⁵ The serotonin theory proposes that increased serotonin or 5-HT_{2A} receptor activity leads to psychosis. The dopamine theory proposes that dopamine is hyperactive in the dopaminergic pathway at dopamine D₂ receptors, causing positive symptoms, which is further supported by the fact that all antipsychotics target blockage of D₂ receptors.⁷⁶ The glutamate theory proposes that the N-methyl-D-aspartate receptor is less functional in the prefrontal cortex.⁷⁵

An early study revealed that administration of lysergic acid diethylamide, a 5-HT antagonist, induced hallucinations, raising the hypothesis that 5-HT deficiency may contribute to the pathogenesis of schizophrenia.⁷⁵ A previous positron emission tomography study on first-episode, drug-naïve patients with schizophrenia revealed lower 5-HT_{2A} receptor binding in the frontal cortex, and 5-HT_{2A} binding was negatively correlated with positive psychotic symptoms in male patients.^{77 78} Therefore, the mechanism may be related to reduced binding of 5-HT to the receptor rather than an absolute decrease in 5-HT. This is further supported by subsequent post-mortem studies that revealed elevated 5-HT and/or its primary metabolite 5-hydroxyindoleacetic acid (5-HIAA) in specific brain regions, although controversial results have been reported.⁷⁹ In the meantime, CSF levels of 5-HIAA have been revealed to be negatively associated with delusions and sadness, as assessed by the Comprehensive Psychopathological Rating Scale but not with suicide attempts.^{80 81}

Early clinical studies also revealed that an amphetamine challenge induced increased dopamine release accompanied by worsening of psychotic symptoms,⁸² and administration of antipsychotic drugs that block D₂ dopamine receptors reduced the severity of prodromal symptoms,⁸³ which indicated that dopamine dysregulation might contribute to the development of psychosis. This concept is further supported by a postmortem study showing the elevation of dopamine and its receptors in brain subregions, including striata, nucleus accumbens and caudate of patients with schizophrenia.⁸⁴ However, a more recent imaging study revealed that reduced dopamine was present in striata and the dorsolateral prefrontal cortex on amphetamine challenge.⁸⁵ Furthermore, CSF levels of homovanillic acid, a dopamine metabolite, are decreased in patients with schizophrenia compared with controls.⁸⁶ Other indirect evidence showed that tyrosine hydroxylase, the rate-limiting enzyme for dopamine synthesis, is elevated in the substantia nigra in patients with schizophrenia compared with healthy controls.⁸⁷ In addition, *in vivo* imaging analysis revealed significant elevation of dopaminergic function in presynapses of the schizophrenia brain, and that the change in presynaptic dopamine function was more consistent in acute psychosis than in chronic disease processes.^{88 89} Other imaging studies

have revealed that the uptake of ^{18}F -dopa is elevated in the striatum and correlated with the severity of psychopathological and neuropsychological impairment in the prodromal phase of the disease.⁹⁰

Glutamate is considered to play a crucial role in the pathophysiology of schizophrenia. Previous meta-analyses on proton magnetic resonance spectroscopy (MRS) studies revealed significant increases in glutamate and glutamine (Glx) in several brain regions, including the basal ganglia, thalamus and medial temporal lobe.⁹¹ In contrast, individual MRS studies revealed reduced levels of glutathione-glutamate components in the anterior cingulate cortex in patients with stable schizophrenia.⁹² In the periphery, lower plasma levels of glutathione have been shown in patients with schizophrenia and are positively correlated with Glx levels in the anterior cingulate cortex as assessed by MRS.⁹³

In addition, other neurotransmitters, such as norepinephrine (NE), have been shown to decrease in serum in schizophrenia compared with controls, and overnight urine levels of NE are negatively correlated with working memory in schizophrenia.^{94,95}

At present, most of the studies are established by *in vivo* imaging or on postmortem brain tissue and occasionally in the CSF. Assessment of neurotransmitters in peripheral biofluids, particularly in the blood, is lacking.

Peripheral protein and metabolite markers

Various peripheral proteins have been assessed in schizophrenia, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), homocysteine (Hcy), vitamin B and G72.

NGF is a neurotrophic protein that regulates various physiological functions. Meta-analyses have revealed that serum and plasma levels of NGF are decreased in both drug-naïve and medicated patients with schizophrenia, independent of demographic factors such as age and sex.^{96–98} This is confirmed by another study of drug-naïve first-episode patients with schizophrenia, indicating that NGF is a stable marker for schizophrenia.⁹⁹ Although serum NGF levels did not differ between schizophrenia and controls in a different study, it was still correlated with grey matter volume in regions such as the left prefrontal lobe, left midcingulate cortex and brainstem in schizophrenia.¹⁰⁰

BDNF is an important neurotrophic factor in the brain. A meta-analysis of drug-naïve first-episode patients with schizophrenia revealed decreased levels of BDNF in the peripheral blood.⁹⁹ An individual study also revealed lower plasma levels of BDNF in schizophrenia and the correlation of BDNF with certain cognitive dimensions, such as memory.¹⁰¹ In addition, patients with schizophrenia and depression present lower plasma levels of BDNF than patients with schizophrenia but without depression, who have lower BDNF levels than those in controls, indicating its potential for predicting clinical manifestations.¹⁰² Importantly, BDNF may change continuously from preclinical to clinical stages, as both

plasma and serum levels of BDNF are highest in patients with chronic schizophrenia (followed by first-episode patients with psychosis, patients and individuals with an at-risk mental state) but do not predict relapse of schizophrenia,¹⁰³ indicating that BDNF may potentially serve as a longitudinal marker to predict progression from at-risk mental state to schizophrenia. Caution should be taken when setting the threshold of BDNF, as sex may be a factor influencing BDNF levels. A previous study showed that plasma levels of BDNF are significantly higher in female patients with schizophrenia than in male patients.¹⁰⁴

Hcy is biosynthesised from methionine, and its abnormal elevation leads to imbalanced neurotransmitters (ie, inhibited synthesis of dopamine, NE and 5-HT), resulting in cognitive impairment and negative symptoms of schizophrenia.¹⁰⁵ Previous studies of a Han Chinese population have shown that the incidence of hyperhomocysteinaemia is more than doubled in patients with schizophrenia compared with controls.¹⁰⁶ Another study of a cohort from Poland showed a similar increased incidence of hyperhomocysteinaemia.¹⁰⁷ Serum levels of homocysteine are elevated as early as the first episode in schizophrenia¹⁰⁸ and are negatively associated with cognitive performance and positively correlated with the Calgary Depression Scale for Schizophrenia.¹⁰⁹ Furthermore, a Japanese study has revealed that total homocysteine-related SNPs are associated with schizophrenia.¹¹⁰ Together, these findings indicate that Hcy may serve as a convenient biomarker in peripheral blood to predict changes in mood and cognition.

Vitamins are considered crucial for body functions. It has been revealed that the level of vitamin B₆ (VitB₆) is decreased in the peripheral blood of patients with schizophrenia compared with controls.^{111,112} This is further supported by a meta-analysis that has shown that lower serum levels of VitB₆ are associated with the risk of schizophrenia.¹¹³ Therefore, VitB₆ may serve as an early marker to assist in predicting the risk of schizophrenia. Findings regarding vitamin B₁₂ (VitB₁₂) are less consistent, with both elevation and reduction of VitB₁₂ levels observed in the peripheral blood of patients with schizophrenia compared with controls.^{111,112,114}

G72 modulates D-amino acid oxidase, which degrades D-serine, which is considered to contribute to glutamate neurotransmission.¹¹⁵ Models built by machine learning revealed that the G72 protein may serve as a potential biomarker for identifying schizophrenia.¹¹⁶ This is consistent with the finding that serum levels of G72 are increased significantly in patients with schizophrenia compared with controls.¹¹⁷ However, CSF analysis failed to show a significant change in G72 levels in patients with schizophrenia compared with controls.¹¹⁵ Furthermore, a meta-analysis revealed that the T-allele of a D-amino acid oxidase activator SNP was associated with schizophrenia in multiple populations, with the G-allele also associated with schizophrenia in an Asian population.¹¹⁸

Gut microbiota and their metabolites

Schizophrenia is associated with reduced microbial diversity and shows a different global community compared with non-psychiatric controls.¹¹⁹ A previous study showed that patients with schizophrenia present more anaerobes.¹²⁰ At the phylum level, 16S rRNA sequencing revealed that patients with schizophrenia present a higher abundance of Proteobacteria compared with controls.¹²¹ However, contrary results are reported in another study showing a reduction of Proteobacteria in schizophrenia.¹²² At the family level, the relative abundance of Christensenellaceae, Enterobacteriaceae and Victivallaceae is increased, whereas that of Pasteurellaceae, Turicibacteraceae, Peptostreptococcaceae, Veillonellaceae, Succinivibrionaceae, Alcaligenaceae, Enterococcaceae, Leuconostocaceae, Rhodocyclaceae and Rikenellaceae is decreased in patients with schizophrenia compared with controls.^{123 124} At the genus level, α and β diversities are increased in schizophrenia.¹²⁰ The relative abundance of *Succinivibrio*, *Megasphaera*, *Collinsella*, *Klebsiella*, *Methanobrevibacter* and *Anaerococcus* is increased, whereas that of *Blautia*, *Coprococcus*, *Roseburia*, *Haemophilus*, *Sutterella* and *Clostridium* is decreased in schizophrenia.^{121 122} At the species level, an increased relative abundance of *Akkermansia muciniphila*, *Bifidobacterium adolescentis*, *Clostridium perfringens*, *Lactobacillus gasseri*, *Megasphaera elsdenii* and *Clostridium coccoides* and a decreased relative abundance of *Bifidobacterium* spp, *Escherichia coli* and *Lactobacillus* spp have been revealed in patients with schizophrenia compared with controls.^{124 125}

Alteration of microbial diversity may reflect the presence of disease and predict the risk and disease stage of schizophrenia. For instance, an increase in Enterobacteriaceae has been shown to be potentially related to an increased risk of schizophrenia, whereas Gammaproteobacteria is associated with a lower risk.¹²⁶ In addition, Lachnospiraceae has been associated with chronic schizophrenia.¹²⁷ Furthermore, the gut microbiota has been shown to differentiate between acute schizophrenia and remission.¹²⁸

Few gut microbiota have been linked to clinical symptoms. For instance, Ruminococcaceae is negatively correlated with the severity of negative symptoms, whereas Bacteroides is positively correlated with depressive symptoms.¹²² Deficit schizophrenia has been shown to present elevated IgA levels in response to *Hafnei alvei*, *Pseudomonas aeruginosa*, *Morganella morganii* and *Klebsiella pneumoniae* compared with non-deficit schizophrenia; more importantly, elevated IgA levels in response to *Pseudomonas* and elevated IgM levels in response to Gram-negative bacteria are highly predictive of deficit schizophrenia.¹²⁹

In addition to the change in gut bacteria, *Candida* has also been shown to be present in patients with schizophrenia. *Candida albicans* seropositivity in schizophrenia has been shown to be correlated with positive psychiatric symptoms, and antibodies against *C. albicans* decreased significantly following probiotic treatment in male patients with schizophrenia.¹³⁰

Pharmacogenomic markers

Pharmacogenomic markers are considered crucial in the treatment of schizophrenia. A majority of antipsychotics are metabolised by cytochrome P450 (CYP450), and genetic polymorphisms may result in poor metabolising capacity, subsequently affecting treatment efficacies and causing side effects. A recent consensus supported the use of pharmacogenomic testing for *CYP450*, *CYP2D6* and *CYP2C19* genes when developing individualised therapeutic strategies and also suggested testing for human leucocyte antigen genes *HLA-A* and *HLA-B* on carbamazepine administration.¹³¹ In addition, when oxcarbazepine is administered, *HLA-B* should be tested; when phenytoin is administered, *CYP2C9* and *HLA-B* should be assessed.¹³¹

In addition to these commonly recognised general genes associated with treatment response, other gene polymorphisms are also important in predicting drug response. A GWAS revealed that higher polygenic risk scores could predict greater post-treatment symptoms, whereas lower polygenic risk scores posed a higher possibility of treatment response.¹³² In a Chinese schizophrenia cohort, polymorphism of the dopamine transporter gene, rs2975226, was associated with allele and genotype frequencies in response to clozapine.¹³³ In addition, polymorphism of serotonergic receptor types 2A, T102C, 2C and 6 has been shown to be correlated with the response to clozapine in schizophrenia.^{134 135} Furthermore, a previous study revealed that polymorphisms of *HTR2C* are associated with the treatment response of *HTR2C* antagonists.¹³⁶

Consensus laboratory process for recognising schizophrenia in clinical practice

Collectively, we proposed a novel laboratory process for diagnosing schizophrenia (figure 1) based on the evidence summarised in this paper (online supplemental table 1). First, although schizophrenia presents strong heritability, attention needs to be paid when using and interpreting genetic markers for diagnostic purposes. CNVs are significant but rare variations; even for the most recognised disease-specific CNV (deletion at 22q11.2), the estimated prevalence is only 1%–2% in schizophrenia. A large number of schizophrenia-associated CNVs have been identified in GWASs; however, validation is required across multiple studies before their use as biomarkers for schizophrenia. Moreover, co-heritability exists between schizophrenia and other mental disorders, including bipolar disorder and major depressive disorder, posing a challenge for differential diagnosis. Epigenetic modulation has attracted increasing research interest in the development of biomarkers. Specific DNA methylation sites and miRNAs have been proposed as potential biomarkers for schizophrenia. Second, inflammatory markers may serve as important biomarkers for predicting (i) disease progression, (ii) treatment response and drug efficiency and (iii) prognosis. Meanwhile, these inflammatory markers are associated with disease mechanisms and thus may be used as biomarkers for mechanism-related research. Third, neurotransmitter markers could potentially be used to assist

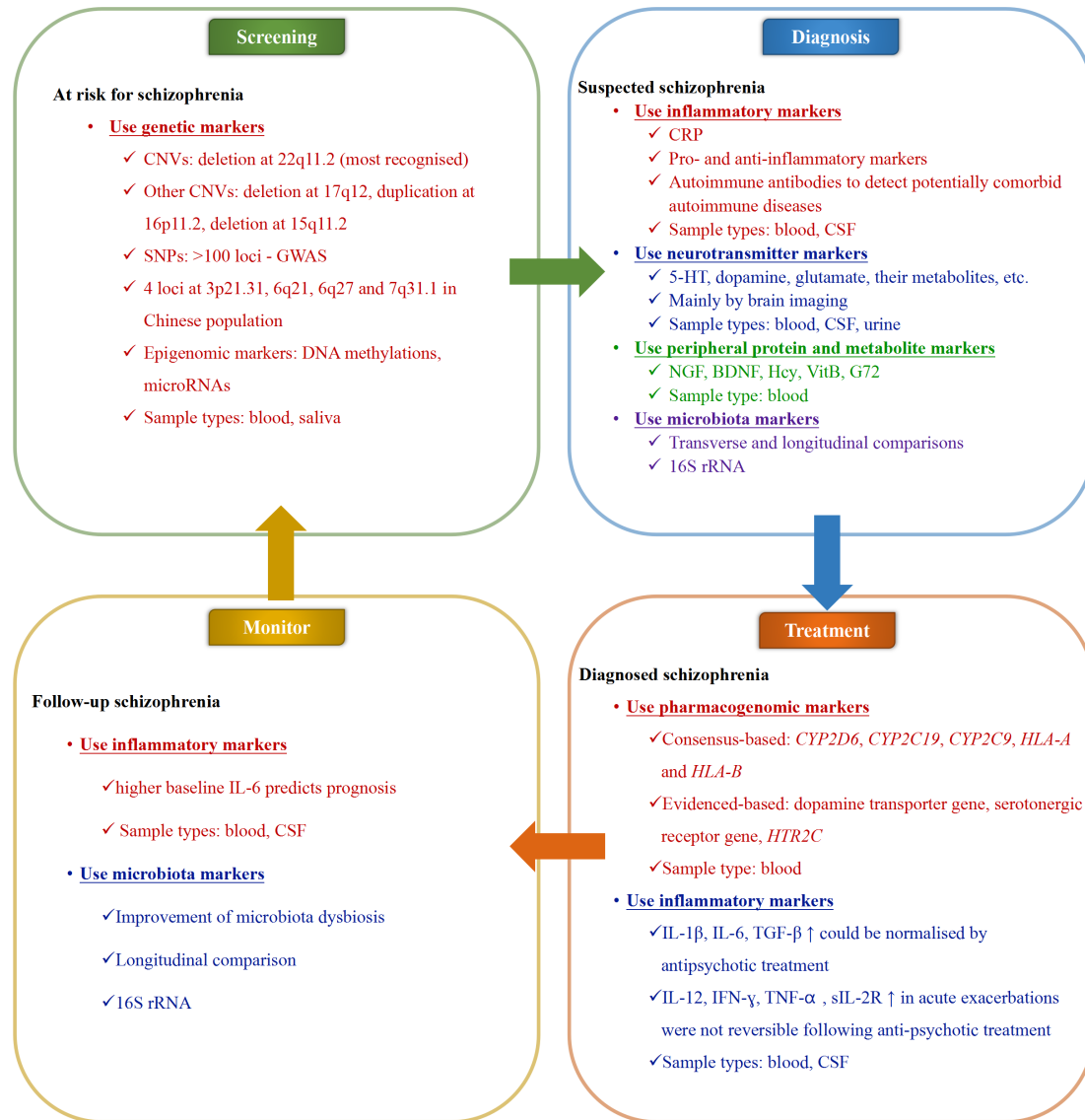


Figure 1 Diagnostic scheme for screening, diagnostic assessments, evaluation of treatment response and postdiagnostic monitoring for schizophrenia. BDNF, brain-derived neurotrophic factor; CNV, copy number variant; CRP, C reactive protein; CSF, cerebrospinal fluid; GWAS, genome-wide association studies; Hcy, homocysteine; HLA, human leucocyte antigen; 5-HT, 5-hydroxytryptamine; IFN, interferon; IL, interleukin; NGF, nerve growth factor; sIL-2R, soluble IL-2 receptor; SNP, single-nucleotide polymorphism; TGF, transforming growth factor; TNF, tumor necrosis factor; VitB, vitamin B.

with the diagnosis of schizophrenia; however, the measurements are mainly performed using imaging analysis, which limits their use in clinical practice. Fourth, peripheral protein markers such as NGF and BDNF could potentially act as stable and longitudinal markers for schizophrenia because changes in these markers are independent of demographic factors and exist continuously from preclinical to clinical stages. In addition, these protein and metabolite markers may reflect certain manifestations; for example, Hcy is negatively associated with cognitive performance. Fifth, alteration of microbial diversity and composition has been observed in multiple studies; therefore, it may reflect the presence of disease and predict the risk and disease stage of schizophrenia. In addition, the gut microbiota has been shown to differentiate between acute schizophrenia and remission and between deficit and non-deficit schizophrenia. Finally,

pharmacogenomic evaluations are critical for individualised treatment strategies, as higher polygenic risk scores predict greater post-treatment symptoms, whereas lower polygenic risk scores indicate a higher possibility of response to treatment. It is noteworthy that polymorphism of the dopamine transporter gene, rs2975226, has been identified in a Chinese schizophrenia cohort as an indicator of responsiveness to clozapine, which may be used as a population-specific marker.

CONCLUSIONS

This study comprehensively and systematically analysed potential biomarkers for identifying schizophrenia, providing a first-hand laboratory testing basis for clinical diagnosis. With the continuous development of new

diagnostic techniques, we believe that more biomarkers will be added to this consensus in the near future.

FUTURE DIRECTIONS

Although genetic risk factors play an important role in developing schizophrenia, further investigations and validations in larger cohorts of certain ethnic groups are required before genetic markers can be used as a diagnostic tool for schizophrenia. This is because novel loci have been identified in a variety of studies, and these loci both overlap and vary across different ethnicities. Epigenetic markers such as DNA methylation sites and miRNAs may be useful biomarkers, but the inconsistency of results generated from different studies makes it difficult to develop specific diagnostic markers. Further investigations are needed to identify schizophrenia-specific genetic markers for precise diagnosis, differential diagnosis and ethnicity-specific markers in the Chinese population. Analysing appropriate biospecimens for accurate detection of genetic markers is also challenging, as peripheral blood mononuclear cells have been used in studies of schizophrenia-associated miRNAs. In future studies, we need to determine an optimised method and sample type to be used for developing diagnostic biomarkers. In terms of neurotransmitters, most of the studies are established by analysing *in vivo* imaging or postmortem brain tissue and occasionally CSF. Assessment of neurotransmitters and their metabolites in peripheral biofluids, particularly in the blood, is lacking. The development of certain novel technologies such as single-molecule array technology and nanobiotechnology is making the clinical use of markers such as disease diagnostic biomarkers possible. In addition, the consistency of the data generated from different studies on the gut microbiota is not satisfactory. In future research, apart from transverse analysis, longitudinal comparison using each individual's data and randomized controlled trials (RCT) are critical for the prediction of disease progression and effectiveness of interventions.

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