REVIEW

Peripheral Biomarkers Revisited: Integrative Profiling of Peripheral Samples for Psychiatric Research

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Peripheral samples, such as blood and skin, have been used for decades in psychiatric research as surrogates for central nervous system samples. Although the validity of the data obtained from peripheral samples has been questioned and other state-of-the-art techniques, such as human brain imaging, genomics, and induced pluripotent stem cells, seem to reduce the value of peripheral cells, accumulating evidence has suggested that revisiting peripheral samples is worthwhile. Here, we re-evaluate the utility of peripheral samples and argue that establishing an understanding of the common signaling and biological processes in the brain and peripheral samples is required for the validity of such models. First, we present an overview of the available types of peripheral cells and describe their advantages and disadvantages. We then briefly summarize the main achievements of omics studies, including epigenome, transcriptome, proteome, and metabolome analyses, as well as the main findings of functional cellular assays, the results of which imply that alterations in neurotransmission, metabolism, the cell cycle, and the immune system may be partially responsible for the pathophysiology of major psychiatric disorders such as schizophrenia. Finally, we discuss the future utility of peripheral samples for the development of biomarkers and tailor-made therapies, such as multimodal assays that are used as a battery of disease and trait pathways and that might be potent and complimentary tools for use in psychiatric research.

Key Words: Biomarker, diagnostic support, functional assay, omics, peripheral sample, tailor-made therapy

Despite extensive recent efforts, the pathogenesis of psychiatric disorders remains poorly understood, mainly because their pathophysiology is not immediately apparent in molecular or histopathologic analyses of the human brain. Psychiatric disorders are diseases of the central nervous system (CNS), and therefore, studies of patient-derived living brain cells provide the most pertinent information. Living brain biopsies from psychiatric patients are rare, and keeping noncancerous tissue alive is arduous because mature neurons do not readily divide. Thus, postmortem brains have been extensively used in brain studies, but these samples are subject to various artifacts that are related to medication, the cause of death, agonal state, and the postmortem interval (1,2). It is therefore difficult to determine whether any observed changes are primary to the disease or are secondary compensatory effects from the protracted disease and its treatment. In addition, obtaining a sufficient number of brains in ideal condition is difficult. New techniques, such as those involving induced pluripotent stem cells (iPSCs), are undoubtedly powerful tools (3,4), and the differentiation of iPSCs into different classes of brain cells provides an opportunity to study neurons from patients and therapeutic response profiles in vitro. Nonetheless, establishing the cells takes several weeks, and genetic instability and diversity exist, even among different clones from one individual. Thus, it is more feasible to obtain peripheral samples that can act as potential biomarkers of disease progression and therapeutic response at different time points. Because psychiatric disorders, such as schizophrenia (SZ) and bipolar disorder (BPD), have genetic components (5), CNS alterations might be reflected in peripheral tissues. Indeed, microarray analyses have found numerous classes of genes that are expressed both in blood and the prefrontal cortex (PFC), including about half of the so-called SZ susceptibility genes (6). Intriguingly, about 50% of genetic variants have been shown to similarly affect transcript abundance in multiple tissues, including blood and brain (7). Furthermore, 22% of the total transcriptome is expressed in both the cerebellum and peripheral blood mononuclear cells with a high intrasubject correlation ($\gamma = .98$) at the genome-wide transcript level, which implies that gene expression in peripheral cells can serve as biomarkers for CNS disease (8). A recent expression quantitative trait loci analysis has also revealed that many cis-acting single nucleotide polymorphisms (SNPs) are shared between blood and brain tissue (9).

However, there are multiple caveats to these preliminary observations, including that the blood and brain samples are not collected from the same subjects, there is little evidence on a global scale of differences in cases and control subjects for blood and brain expression, the evidence for single gene differences in both brain and blood is mixed and not corrected for genome-wide correlations, and the fold change is often discordant in blood-brain comparisons. Notwithstanding these limitations, a small number of genes have been shown to be identically altered in both the brain and blood of patients with autism (10). As for epigenetic variations, tissue-dependent epigenetic markers are now being investigated, and a list of candidate genes for peripheral biomarkers of diseases has been compiled (11,12). Finally, one of the biggest advantages of peripheral sample cells is that they can be used for functional cellular analyses and the direct evaluation of dynamic cellular responses underlying various cellular events, which will be discussed in the section Functional Cellular Assays of Peripheral Samples.

Despite the advantages of peripheral samples as surrogates for CNS samples, discouraging results suggesting limited commonalities among different tissue types have been reported (13), and

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it is crucial to determine which signaling pathways and biological processes are, and are not, conserved between CNS and peripheral samples. Peripheral samples should be used as surrogates only when common signaling pathways or biological processes are observed in the two types of samples. Table 1 shows the commonalities and/or differences between peripheral samples and the CNS. Peripheral signaling that is parallel with that in the CNS could unveil the pathophysiology of disease as well as future candidates for biomarkers.

In this review, we present an overview of the use of peripheral samples in classical techniques and for the development of disease models and biomarkers. A summary of all findings is beyond the scope of this article, and we thus focus on the following three categories of samples: 1) freshly prepared primary cells, including red blood cells (RBCs), platelets, and lymphocytes; 2) primary cells with cell line-like features, including lymphoblastoid cell lines (LCLs) and fibroblasts; and 3) biofluids, including serum and plasma. Our review does not include olfactory epithelium cells, despite their usefulness, because they are considered neuronal cells and their sampling accessibility is relatively difficult, in contrast to peripheral samples.

Available Types of Peripheral Samples

Many primary cells tend to be highly differentiated, and they may lack many of the signaling pathways and biological processes that are present in the CNS. Even shared signaling pathways might function differently in different biological contexts. Thus, an empirical criterion for the use of primary cells is whether they share pathways or processes similar to those of the CNS cells of interest. Primary cells can be affected by presampling factors, such as health status, medication use, smoking, diet, and circadian rhythms. In contrast, cell line-like samples could potentially be free from environmental and state-related changes after a certain number of passages. The advantages of cell lines include the opportunity to obtain large amounts of molecular material and their ability to be cryopreserved in liquid nitrogen for later use. The characteristics of several types of freshly prepared cells, as well as primary cells, are briefly summarized in the following sections.

Peripheral Blood Cells. Peripheral blood is composed of fluidic (serum) and cellular components, which are collected by density-gradient centrifugation. Genomic DNA, RNA, and proteins that are extracted from cellular components are routinely used for genetic and biological studies. These cells and particularly lymphocytes are a major source of cytokines, and various hypotheses regarding the relationship between cytokine signaling and psychiatric disorders have been tested. For example, cytokines alter CNS functions that mediate behavioral responses, and the CNS, in turn, regulates lymphocyte metabolism (14,15). Lymphocytes express a broad repertoire of receptors for cytokines, neuroendocrine hormones, and neuropeptides, including glucocorticoid, mineralocorticoid, brain-derived neurotrophic factor, dopamine (D2, D3, and D5), and muscarinic and nicotinic acetylcholinergic, serotoninergic, gamma-aminobutyric acidergic, cannabinoid, prolactin, and somatostatin receptors (16). A model of the complicated bidirectional communications between the CNS and non-CNS systems has been proposed (see Functional Cellular Assays of Peripheral Samples).

Platelets are irregularly shaped anucleate cells that are easily isolated by centrifugation from platelet-rich plasma. They express a broad range of neurotransmitter receptors and transporters (17). Therefore, it is not surprising that platelets have been extensively used to examine the functional properties of receptors and transporter activities (see Neurotransmission). Some indexes of platelets have been correlated with those of the CNS (Table 1); for example, mitochondrial complex I activity in platelets has been correlated with cerebral glucose utilization (18) and the severity of positive symptoms in patients with SZ (19). Neuroleptics have been found to induce parallel changes in the expression levels of serotonin and dopamine receptors in the CNS and in platelets (20). These findings support the use of platelets in the study of neurotransmitter receptor function in psychiatric disorders.

Mature human RBCs are also anucleate and thus cannot synthesize RNA. However, because all cell plasma membranes comprise a large number of common phospholipids, RBCs have frequently been used to study lipid metabolism (see Metabolism).

Serum/plasma has been frequently used for biomarker development (see Disease Modeling and Biomarkers). Interestingly, cell-free circulating nucleic acids are present in serum, in which RNAs and microRNAs are very stable. Some RNAs and microRNAs are contained in small vesicles called exosomes, and they are secreted from distant cells and tissues, including the brain (21).

LCLs. Infection by the Epstein-Barr virus transforms human resting B cells into actively proliferating LCLs 1 month after infection (22). LCLs have several advantages over other types of cells. They proliferate rapidly and are nearly immortal. Thus, they can serve as an unlimited source of biomaterial and can be stored in liquid nitrogen. After re-establishment in culture (23), cells have been repeatedly used for multiple assays, including cellular functional assays (24). For instance, Sei *et al.* (25) have assessed the induction of cell migration by neuregulin1 (NRG1), which is a promising SZ susceptibility gene, in LCLs from patients with SZ, and they have found that NRG1-induced migration is significantly decreased compared with that of control individuals. Cell migration is highly influenced by *NRG1* polymorphisms and epistatic interactions of *NRG1* with other SZ genes, clearly suggesting the benefit of assessing cellular events in combination with genetic variations for more than one SZ gene.

Fibroblasts. Skin fibroblast cultures can be easily established without any transformation process, and they can usually be maintained through approximately 20 passages. However, the age of the patients that can be examined is limited because fibroblasts from patients who are older than 50 years show shorter life spans in culture than cells from younger patients (26). The greatest advantage of fibroblasts may be their undifferentiated state, which is free from state-related changes such as those related to diet, hormones, and drugs. Because fibroblasts exhibit a broad range of genes that are involved in biological signaling, various processes in fibroblasts have been extensively studied, including genomic regulatory mechanisms, the cell cycle, cell adhesion, metabolism (glucose, lipid, and serine), neurotransmission, neurotrophin receptor-mediated signal transduction (adrenergic, cholinergic, and serotonergic), tyrosine transport capability, and cellular antioxidant defense (27–31).

Omics Assays of Peripheral Samples

Omics Approaches and Effects of Genotype

Due to innovations in analytical techniques, omics approaches, in which all constituents are measured collectively at the same molecular level, have been extensively used. In this section, we review the results of epigenome, genome, transcriptome, proteome, and metabolome assays of peripheral samples, and a comprehensive list is shown in Table S1 in Supplement 1. Omics data can be influenced by several factors, including genetic

Table 1. Similarities and/or Differences between Peripheral Sample and CNS Samples

	Sampling							References	
Disease	Periphery	CNS	From Same Individuals	Research Design/Focus	Experimental Method	Findings	Similarities between CNS and Periphery	Year, Journal	PMID
Genomics									
BPD	LCLs	Postmortem brain (DLPFC)	No	An unbiased genome-wide	DNA microarray	Differential expressions of PDLIM5 and HSPF1 both in blood and brain of BPD	Two genes, out of 10 genes identified in postmortem, were replicated in LCLs	2004, *Mol Psychiatry*	14743183
SZ	Blood	Postmortem brain (DLPFC)	No	An unbiased genome-wide	DNA microarray	Upregulation of SELENBP1 both in blood and brain of SZ	Six genes, out of 177 genes identified in postmortem, were replicated in blood	2005, *Proc Natl Acad Sci U S A*	16223876
Control	Blood	Postmortem brain (Multiple regions)	No	An unbiased genome-wide	DNA microarray	(1) Similarity of some signaling between blood/PFC (2) Half of SZ genes expressed both in blood/PFC	Some signaling were similar	2006, *Am J Med Genet B Neuropsychiatr Genet*	16526044
SZ/Control	Lymphocyte	Postmortem brain (PFC)	No	HTR2A	RT-PCR	Disease associated SNP 102C is expressed lower than 102T in brains	Lymphocyte: monoallelic, brain: biallelic expression	2006, *Biol Psychiatry*	17069769
Control	Blood	Meta-analysis data	No	An unbiased genome-wide	DNA microarray	Weak correlations between mean expression between brain and blood	Only some signaling were similar	2010, *BMC Genomics*	20961428
Control	Postmortem blood	Postmortem brain (cerebellum)	Yes	An unbiased genome-wide	DNA microarray	22% of total transcriptome expressed in both cerebellum and blood with high correlation ($\gamma = .98$).	20% genes were expressed at similar level between blood and brain	2010, *Am J Med Genet B Neuropsychiatr Genet*	20127885
SZ	Blood (discordant MZ twins)	Postmortem brain	No	An unbiased methylome-wide	Methylation microarray	Hypomethylation of ST6GALNAC1 both in blood and brain of SZ	Brain DNA methylation were higher (~85%) than the blood (~40%)	2011, *Hum Mol Genet*	21908516
BPD	LCLs	Postmortem brain (PFC)	No	An unbiased promoter-wide	Promoter tiling array and pyrosequencing	Hypermethylation of SLC6A4 both in blood and brain of BPD		2011, *Transl Psychiatry*	22832526
BPD	Blood and sperm	Postmortem brain	No	HCG9	Bisulfite sequence	Hypomethylation of HCG9 in all tissues of BPD	HCG9 methylation profiles were similar across tissues	2012, *Mol Psychiatry*	21647149
Control	Blood (before death)	Postmortem brain (multiple regions)	Yes	An unbiased methylome-wide	Methylated DNA immunoprecipitation	Highly distinct patterns of DNA methylation between CNS and blood	Methylation was generally tissue-specific, while that of CpG-rich promoter is largely similar	2012, *Genome Biol*	22703893
SZ/Control	Blood	Postmortem brain	No	An unbiased methylome-wide	Methylation microarray	Methylation levels were less variable than gene expression	Moderate similarity of age-related CpG methylation ($\gamma = .33$)	2012, *Genome Biol*	23034122

Table 1. (continued)

Disease	Sampling: Periphery	Sampling: CNS	Sampling: From Same Individuals	Research Design/Focus	Experimental Method	Findings	Similarities between CNS and Periphery	References: Year, Journal	References: PMID
Proteomics/Metabolics									
Control (SIDS)	Blood	Cortex, retina	Yes	Fatty acid composition	Gas chromatography	Breast-fed infants had a higher DHA% both in brain and RBC	Similar DHA% between brain/RBC ($\gamma = .33$)	1994, *Am J Clin Nutr*	7913291
SZ/BPD/ Control	RBC	Postmortem brain (DLPFC)	No	Lipid composition	Mass spectrometry	Alterations in the free fatty acids and ceramide both in blood and brain of SZ and BPD group	Closely correlated but quantitatively different between brain and RBC	2008, *J Proteome Res*	18778095
SZ	RBC, serum, and liver	Postmortem brain, CSF	Partially Yes	Proteomic profiling	Mass spectroscopy and 2D-DIGE	Downregulation of ApoA1 in all 5 tissues of SZ	No correlation of CSF and serum apoA1 levels from the same subjects	2008, *Mol Psychiatry*	17938634
SZ	Serum	Postmortem brain (BA 10)	No	Proteomic/ metabolic profiling	Multiplex immunoassay panel (187 molecules)	21 analytes were altered in SZ postmortem brain	5 analytes showed same directional change both tissues	2012, *PLoS One*	23118852
Functional Cellular Assay									
SZ	Skin fibroblast	Multiple regions	Yes	Comparison of tyrosine kinetics	▪ Uptake of tyrosine (fibroblast) ▪ Tyrosine transport across BBB (PET)	Utilization of tyrosine was lower in the SZ (PET), and tyrosine transport was decreased in the fibroblast	Similar tyrosine kinetics between tissues	1991, *J Nucl Med;* 1994, *Schizophr Res*	1941137 7841139
MDD	Platelet	CSF	Yes	Comparison of 5-HT indexes	▪ 5-HT index (platelet) ▪ 5-HT (CSF)	No correlation between platelet and CSF for 5-HT indexes	No support for the use of platelet for CSF 5-HT monitoring	1992, *Arch Gen Psychiatry*	1376106
SZ (first episode, drug naïve)	RBC	Living brain	Yes	Comparison of lipid metabolism	▪ Fatty acids (RBC) ▪ 31P-MRS (brain)	A parallel decrease in RBC phospholipid fatty acids and phospholipid metabolism in the PFC of SZ individuals	Similarity between RBC and PFC lipid metabolism ($\gamma = .56$)	2002, *Biol Psychiatry*	12372654
Control	Plasma	mPFC	Yes	Monitoring the brain Glu by plasma	▪ HPLC (plasma) ▪ 1H-MRS (brain)	No correlation between plasma and mPFC for Glu and Glx (Glu + Gln) concentration	No support for the use of blood for CNS Glu monitoring	2006, *J Psychiatry Neurosci*	17136218
SZ	Platelet	Living brain	Yes	Platelet as a predictor of CNS function	▪ Mitochondrial complex I (platelet) ▪ FDG-PET (brain)	Complex I activity in platelet was correlated with PANSS and cerebral glucose metabolism	Similarity between peripheral and cerebral energy metabolism	2007, *Prog Neuropsycho Biol Psychiatry*	17329000
MDD/BPD	Plasma	Living brain	Yes	Monitoring the brain PUFAs level by plasma	▪ PUFA (plasma) ▪ FDG-PET (brain)	Temporoparietal glucose usage correlated positively with both DHA% and AA%	Similar PUFA ratio between plasma and cortex ($\gamma = .77$)	2009, *Prostaglandins Leukot Essent Fatty Acids*	19128951

2D-DIGE, two-dimensional difference gel electrophoresis; 5-HT, serotonin; AA, arachidonic acid; BA, Brodmann area; BBB, blood-brain barrier; BPD, bipolar disorder; CNS, central nervous system; CSF, cerebrospinal fluid; DHA, docosahexaenoic acid; DLPFC, dorsolateral prefrontal cortex; FDG, fluorodeoxyglucose; Gln, glutamine; Glu, glutamate; Glx, glutamate + glutamine; HPLC, high-pressure liquid chromatography; LCLs, lymphoblastoid cell lines; mPFC, medial prefrontal cortex; MDD, major depressive disorder; MRS, magnetic resonance spectroscopy; MZ, monozygotic; PANSS, Positive and Negative Syndrome Scale; PBMC, peripheral blood mononuclear cell; PET, positron emission tomography; PFC, prefrontal cortex; PMID, PubMed identifier; PUFA, polyunsaturated fatty acids; RBC, red blood cells; RT-PCR, reverse transcriptase polymerase chain reaction; SIDS, sudden infant death syndrome; SZ, schizophrenia.

background, environmental factors, and stochastic events. Especially, epigenome and transcriptome information are known to be considerably influenced by the genotype of each individual. Many expression quantitative trait loci analyses have revealed that genomic variations within different ethnic groups can account for a substantial proportion of transcriptome variations. In the simple case, cis-located SNPs can affect transcription regulation by making or disrupting transcription factor binding sites. For the epigenome, SNPs that locate CpG sites disrupt cytosine DNA methylation, whereas those locating DpG or CpH create new methylation sites when SNPs form CpG sequences. Through the extensive comparison of the whole genome DNA methylation statuses of various tissues and cell lines, differentially methylated regions in the human genome have been reported to contain SNPs identified by various genome-wide association studies (32). Interestingly, differentially methylated regions in blood cells contain SNPs that are related to neurological and behavioral disorders, ensuring the importance of epigenome studies of peripheral blood samples. Numerous SNPs can affect proteome and metabolome information, which can be used to screen SNPs or mutations that have severe functional significance, including protein conformational changes or the accumulation or reduction of specific metabolites.

DNA Methylation. Aberrant DNA methylation leads to a number of diseases, including cancer and mental retardation (33), and environmental insults have been reported to result in brain epigenetic alterations, which are associated with behavioral changes in animal models (34). Although epigenetic studies have usually been performed on postmortem brain tissue (35,36), similar disease-associated DNA methylation changes at specific genomic regions have also been detected in peripheral samples (37,38). This is a strong advantage of peripheral tissues compared with iPSCs because epigenetic markers are thought to be erased in reprogrammed cells. Global DNA hypomethylation has been repeatedly observed in peripheral leukocytes from patients with SZ, particularly patients with early-onset SZ (39) and male patients (40). Although the pathophysiological significance of DNA hypomethylation remains unclear, it is known to increase mutation rates and chromosomal instability (41), implicating disturbances in the mechanisms of genome integrity and maintenance in SZ. The epigenetic states of various SZ candidate genes have also been frequently examined in peripheral blood, saliva, and LCLs (36). It should be noted that the reported changes in DNA methylation levels are subtle (generally $<5\%$), and thus, their pathophysiological role in psychiatric disorders is not clear. In addition, whether epigenetic changes are stable over time in peripheral tissues remains unclear. However, the number of reports of significant alterations in DNA methylation in peripheral cells has been increasing, and this research area provides new opportunities for studying psychiatric disorders.

Gene Expression. Transcriptome analysis techniques, including DNA microarrays and next-generation sequencing, have been improving continuously. Array results have been generally difficult to compare and replicate because of platform and statistical differences (42,43). However, data analyses that have focused on gene ontology have been used to compare studies. The early-stage studies of gene expression in peripheral samples have been reviewed elsewhere (44). Comprehensive array technologies have frequently been used to explore gene expression patterns to discriminate disease and disease subtypes from control subjects. For example, Tsuang *et al.* (45) have suggested that eight genes in peripheral cells are useful markers for the discrimination of patients with SZ and BPD from control subjects. The expression profile of 14 genes in peripheral blood cells has also been used to discriminate between patients with SZ and normal control subjects with an accuracy of 87.9% (46). Although there have been many efforts to identify objective gene expression-based markers for psychiatric diagnoses, the identified genes rarely overlap across studies, and attempts to replicate previous findings in different cohorts have generally yielded disappointing results (47).

Gene expression analyses within a well-characterized family or among discordant siblings may provide complementary information that would improve our understanding of risk and protective gene expression changes in individuals. Indeed, analyses of gene expression in blood samples from patients with SZ and their unaffected siblings, as well as unrelated control subjects, have identified expression changes that are shared between patients and their siblings and patient- or sibling-specific expression changes (48,49). Similarly, Petryshen *et al.* (50,51) have examined gene expression profiles in blood samples from discordant SZ siblings and identified genetic associations and concomitant alterations in the expression of several genes, including *SMDF* (a transcription variant of *NRG1*) and *GABRB6*. Another excellent example of the utilization of gene expression dynamics in peripheral cells is studies of circadian rhythms. Yang *et al.* (52) have observed the altered expression of the clock-related genes *DEC2* and *DBP* and a reduced amplitude of the rhythmic expression of *BMAL1*, *REV-ERBalpha*, and *DBP* in fibroblasts of patients with BPD. Because cultured fibroblasts have a circadian clock that is comparable with that of the suprachiasmatic nucleus, clock signaling in fibroblasts may be a good model of the circadian disturbances that have been observed in patients with BPD.

Protein and Metabolite Levels. Molecular profiles that are obtained by proteome and metabolome analyses would be useful for developing biomarkers and diagnostic purposes. For example, proteomic investigations of serum and RBCs from first-onset SZ patients, as well as those of postmortem brain and liver tissues in a different cohort, have revealed that ApoA1 downregulation was common to all five sample types (53). The downregulation of apolipoproteins, including ApoA1, in the serum of patients with SZ and BPD has also been reported in other proteomic studies (54,55). Note, however, that the proteome and metabolome results are highly dependent on their techniques, because there are many different methods for sample preparation and analyte detection. Thus, there is much room for employing these approaches in analyzing peripheral tissues. The technical aspects and potential pitfalls have been the subject of excellent reviews elsewhere (56,57).

Functional Cellular Assays of Peripheral Samples

Neurodevelopment and later plasticity involve continuous cellular responses to various stimuli, including neurotransmitters and neurotrophins, as well as to damaging stimuli. Therefore, direct investigations of dynamic cellular responses to stimuli would provide potential insights into psychiatric disorders. Table S2 in Supplement 1 lists studies of the cellular dynamics of SZ individuals. Among the responses, we focus on cell signaling pathways that are common to the CNS and peripheral cells and that could be a powerful cellular model of functional assays in this section.

Neurotransmission. Human peripheral cells express several neurotransmitter receptors, including the *N*-methyl-D-aspartate

(NMDA)- and alpha-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-type glutamate receptor and the associated second messenger molecules (16). Thus, peripheral cells have been used as a peripheral model of central synaptosomes. Indeed, platelets have been extensively used as models of central synaptosomes because they have been shown to accumulate glutamate and serotonin-like synaptosomal preparations (28,58). Glutamate-stimulated $[Ca^{++}]_i$ responses are reduced in the platelets of patients with SZ (59), which is similar to the findings of a recent report that suggested that relative NMDA receptor binding in the left hippocampus of medication-free SZ patients is significantly reduced compared with that in healthy subjects, as indicated by studies with a selective single-photon emission tomography NMDA receptor tracer (60). Considering that glutamatergic signaling dysregulation may contribute to SZ pathophysiology (61) and that some glutamate subunit genes have been shown to be positively associated with SZ (61), the use of platelets as a peripheral surrogate for glutamatergic transmission may be useful for functional studies.

Metabolism. A high prevalence of impaired glucose tolerance has been reproducibly reported in patients with SZ, even in drug-naive patients and first-degree relatives of affected individuals (62). Neuroimaging studies with positron emission tomography (PET) and magnetic resonance spectroscopy have indicated metabolic alterations in several brain regions in subjects with SZ (63,64). A parallel transcriptomic, proteomic, and metabolomic approach to SZ brain tissue has suggested that half of the proteins that have been identified to be altered by proteomic analysis are associated with mitochondrial function and oxidative stress responses, and this result is mirrored by transcriptional and metabolic perturbations (65), thus implying altered cerebral energy metabolism and mitochondrial dysfunction in the pathophysiology of SZ. Mitochondrial complex I activity in platelets has been shown to be correlated with PET cerebral glucose utilization (18) and psychiatric severity (19).

Perturbed lipid metabolism has been suggested in SZ. Lipids make up over half of the brain's dry weight, and thus, even small changes in key fatty acids can lead to a broad range of membrane dysfunctions, which may be particularly important during neurodevelopment because cell proliferation, neurite outgrowth, and synaptogenesis involve the dynamic synthesis and breakdown of phospholipids. In several conditions, including Down syndrome, lipid metabolism has been reported as abnormal in both neural and peripheral tissues (66), suggesting that membrane abnormalities are present in both neural and peripheral tissues in psychiatric conditions. Indeed, a variety of deficits in the metabolism of lipids in the RBCs and platelets of patients with SZ have been reported. Free fatty acids have been shown to be significantly decreased in the postmortem PFC of patients with SZ and the RBCs of living SZ patients (67–69). Six double-blind placebo-controlled studies of the use of eicosapentaenoic acid, which is a polyunsaturated fatty acid, for the treatment of SZ have been conducted, and four studies showed that eicosapentaenoic acid had clinical benefits in patients with SZ (70,71). Notably, clinical improvement is positively correlated with an increase in RBC lipid concentration (72), suggesting that RBC lipid concentration may serve as a biomarker of therapeutic efficacy. A significant correlation between RBC membrane fatty acids and in vivo brain phospholipid metabolite levels, as measured by multivoxel ^{31}P-magnetic resonance spectroscopy, has been consistently observed in the PFC of patients of SZ (68). However, the lack of correlations with other brain regions suggests that these results require further validation.

Cell Cycle and Apoptosis. Unbiased gene expression analyses have suggested that cell cycle-related pathways are significantly affected in postmortem SZ brains (73). At all stages of the cell cycle, if DNA has been irreversibly damaged, cells normally undergo apoptosis. It has been estimated that, depending on the region, 20% to 80% of all neurons that are formed in the CNS undergo apoptosis during development. Thus, apoptosis and cell cycle alterations may be underlying mechanisms of the neurodevelopment of SZ (31,74,75). This possibility is supported by reports that p53, which is an apoptosis-related gene, is a susceptibility gene for SZ (76) and the reduced incidence rates of smoking-unrelated cancers in an epidemiological meta-analysis of SZ patients and their first-degree relatives (77). This reduced incidence rate suggests that a putative cell cycle disturbance might be observable in both the peripheral tissue and the brain. An excellent study of fibroblasts has suggested that the cell cycle is abnormal in individuals with SZ: fibroblasts from first-episode, drug-naive SZ patients take longer to establish initial growth and have prolonged doubling time compared with fibroblasts from control subjects (78). Intriguingly, the prolonged doubling time has been significantly associated with poorer premorbid social functioning during childhood (78). In addition, disrupted fibroblast responses to growth factors in first-episode, drug-naive psychotic patients have been observed (79).

Immune System. Many autoimmune diseases are more prevalent among patients with SZ, suggesting the involvement of immune-related dysfunction in SZ (80). Five genome-wide association studies of SZ have provided converging evidence for an association between SZ and the major histocompatibility complex (81). Many studies have examined the influence of various mitogens and neurotransmitters on cytokine secretion in lymphocytes, and a meta-analysis of 62 original studies that measured cytokines in blood samples from patients with SZ has verified a significant increase in the concentrations of interleukin 1 receptor antagonist, soluble interleukin 2 receptor, and interleukin 6 (82,83). Histopathologic and PET evidence for the pathologic activation of microglia in SZ individuals is accumulating. Fate-mapping studies have shown that microglia and macrophages arise from a common primitive macrophage precursor, and gene expression profiling has shown a close relationship between bone marrow-derived macrophages and microglia in C57BL/6 mice (84). Because peripheral blood cells represent major cellular components of the immune system, they could be an appropriate template for the assessment of immune-related cellular dynamics.

Disease Modeling and Biomarkers

The diagnoses of psychiatric disorders depend solely on symptomatic information due to the lack of objective biomarkers. Thus, the establishment of practical state- and trait-dependent biomarkers in patient-derived peripheral samples is highly desirable. However, given that psychiatric disorders, including SZ, are highly heterogeneous and multifunctional, the discovery of a single biomarker with high specificity and sensitivity is unlikely. Of note, the first blood-based diagnostic aid for SZ, VeriPsych, was launched in 2010. This approach is based on a multiplex immunoassay format involving the simultaneous measurement of different protein and hormone biomarkers with implementation of an algorithm for a mathematical decision rule (85). Further refinement of the targeted molecules and the algorithm is now underway and highly anticipated.

The other possible application of peripheral biomarkers is for clinical predictions, such as prognosis, relapse, drug response, and suicidal attempts. Because these predictions need to be easily and repeatedly measurable with a high degree of reproducibility, against reasonable costs, easy accessibility of the peripheral sample for the predictive biomarker would be highly appreciated for the clinical environment. Exploratory attempts have been extensively performed with peripheral samples (Table S3 in Supplement 1). Interestingly, significant differences have been reported in blood-based molecular signatures at the last clinical visit before relapse, implying their use as possible predictors that help in clinical decisions to avoid relapse (86). Suicide is among the top 10 leading causes of death in individuals of all ages, and increased impulsivity is highly related to the transition from suicidal ideation to attempt. Even though impulsivity involves a heterogeneous repertoire of factors and potentially overlapping neurobiological substrates, they may be less genetically complex and more easily assessed than the psychiatric disorder itself. A summary of biological markers that link to future suicidal behavior has been made, but it is still challenging to find both promising and easily assessable peripheral predictors of suicide (Table S3 in Supplement 1). In the future, it will be useful to examine multiple tests and risk factors, including peripheral markers, together with cerebrospinal fluid samples, brain imaging, and the patient history of attempted suicide in the prediction of suicide risk. Likewise, examining multiplex cellular responses to well-designed stimuli might provide predictions of other clinical variables, and these objective biomonitoring methods for affected individuals would enable personalized therapeutic strategies with heuristic value.

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