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 may 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 (γ = .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
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, pro-lactin, 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.

Many 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, neurotransphin receptor-mediated signal transduction (adrenergic, cholinergic, and serotonergic), tyrosine transport capability, and cellular antioxidant defense (27–31).
<table>
<thead>
<tr>
<th>Disease</th>
<th>From Same Individuals</th>
<th>Research Design/Focus</th>
<th>Experimental Method</th>
<th>Findings</th>
<th>Similarities between CNS and Periphery</th>
<th>Year, Journal, PMID</th>
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</thead>
<tbody>
<tr>
<td>Genomics</td>
<td>LCLs</td>
<td>No</td>
<td>An unbiased genome-wide DNA microarray</td>
<td>Differential expressions of PDLIM5 and HSPF1 both in blood and brain of BPD</td>
<td>Two genes, out of 10 genes identified in postmortem, were replicated in LCLs</td>
<td>2004, Mol Psychiatry 14743183</td>
</tr>
<tr>
<td>BPD</td>
<td>Blood</td>
<td>No</td>
<td>An unbiased genome-wide DNA microarray</td>
<td>Upregulation of SELENBP1 both in blood and brain of SZ</td>
<td>Six genes, out of 177 genes identified in postmortem, were replicated in blood</td>
<td>2005, Proc Natl Acad Sci U S A 16223876</td>
</tr>
<tr>
<td>Control</td>
<td>Blood</td>
<td>No</td>
<td>An unbiased genome-wide DNA microarray</td>
<td>(1) Similarity of some signaling between blood/PFC</td>
<td>Some signaling were similar</td>
<td>2006, Am J Med Genet B Neuropsychiatr Genet 16526044</td>
</tr>
<tr>
<td>SZ/Control</td>
<td>Lymphocyte</td>
<td>No</td>
<td>HTR2A RT-PCR</td>
<td>Disease associated SNP 102C is expressed lower than 102T in brains</td>
<td>Lymphocyte: monoallelic, brain: biallelic expression</td>
<td>2006, Biol Psychiatry 17069769</td>
</tr>
<tr>
<td>Control</td>
<td>Blood</td>
<td>No</td>
<td>An unbiased genome-wide DNA microarray</td>
<td>Weak correlations between mean expression between brain and blood</td>
<td>Only some signaling were similar</td>
<td>2010, BMC Genomics 20961428</td>
</tr>
<tr>
<td>Control</td>
<td>Postmortem blood</td>
<td>Yes</td>
<td>An unbiased genome-wide DNA microarray</td>
<td>22% of total transcriptome expressed in both cerebellum and blood with high correlation ($\gamma = .98$)</td>
<td>20% genes were expressed at similar level between blood and brain</td>
<td>2010, Am J Med Genet B Neuropsychiatr Genet 20127885</td>
</tr>
<tr>
<td>SZ</td>
<td>Blood (discordant MZ twins)</td>
<td>No</td>
<td>An unbiased methylome-wide DNA microarray</td>
<td>Hypomethylation of ST6GALNAC1 both in blood and brain of SZ</td>
<td>Brain DNA methylation were higher (~85%) than the blood (~40%)</td>
<td>2011, Hum Mol Genet 21908516</td>
</tr>
<tr>
<td>BPD</td>
<td>LCLs</td>
<td>No</td>
<td>An unbiased promoter-wide Promoter tiling array and pyrosequencing</td>
<td>Hypermethylation of SLC6A4 both in blood and brain of BPD</td>
<td>HCG9 methylation profiles were similar across tissues</td>
<td>2012, Mol Psychiatry 21647149</td>
</tr>
<tr>
<td>BPD</td>
<td>Blood and sperm</td>
<td>No</td>
<td>HCG9 Bisulfite sequence</td>
<td>Hypomethylation of HCG9 in all tissues of BPD</td>
<td>HCG9 methylation was generally tissue-specific, while that of CpG-rich promoter is largely similar</td>
<td>2012, Genome Biol 22703893</td>
</tr>
<tr>
<td>Control</td>
<td>Blood (before death)</td>
<td>Yes</td>
<td>An unbiased methylome-wide Methylated DNA immunoprecipitation</td>
<td>Highly distinct patterns of DNA methylation between CNS and blood</td>
<td>Methylation was generally tissue-specific, while that of CpG-rich promoter is largely similar</td>
<td>2012, Genome Biol 23034122</td>
</tr>
<tr>
<td>SZ/Control</td>
<td>Blood</td>
<td>No</td>
<td>An unbiased methylome-wide Methylation microarray</td>
<td>Methylation levels were less variable than gene expression</td>
<td>Moderate similarity of age-related CpG methylation ($\gamma = .33$)</td>
<td>2012, Genome Biol 23034122</td>
</tr>
</tbody>
</table>
### Table 1.

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

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; Glu, 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; PUFAs, 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. Espe-
cially, 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
epigene, 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
status 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 behav-
ioral 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 signifi-
cance of DNA hypomethylation remains unclear, it is known to
increase mutation rates and chromosomal instability (41),
implying disturbances in the mechanisms of genome integrity
and maintenance in SZ. The epigenetic status of various SZ
candidate genes have also been frequently examined in periph-
eral blood, saliva, and LCLs (36). It should be noted that the
reported changes in DNA methylation levels are subtle (gen-
erally <5%), and thus, their pathophysiological role in psychi-
atriat 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 alter-
ations in DNA methylation in peripheral cells has been increas-
ing, and this research area provides new opportunities for
studying psychiatric disorders.

Gene Expression. Transcriptome analysis techniques, includ-
ing DNA microarrays and next-generation sequencing, have been
improving continuously. Array results have been generally diffi-
cult 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, Tsuch 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
among discordant siblings may provide complementary informa-
tion 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 GABBR8. 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-ERBalp, 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 signal-
ing 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-naïve 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 polysaturated 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-naïve 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-naïve 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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