**Schizophrenia Bulletin** vol. 35 no. 6 pp. 1031–1033, 2009
doi:10.1093/schbul/sbp106
Advance Access publication on September 25, 2009

**EDITORIAL**

# Postmortem Brain Tissue for Drug Discovery in Psychiatric Research

**Sanghyeon Kim[1,2] and Maree J. Webster[2]**

[2]Stanley Brain Research Laboratory, Stanley Medical Research Institute, 9800 Medical Center Drive, Rockville, MD 20850

Schizophrenia, bipolar disorder, and severe depression are common and extremely disabling diseases thought to be caused by an interaction of genetic and environmental factors. Medications currently available to treat these diseases produce varying degrees of symptom amelioration in most patients but often cause unwanted side effects. The need for more effective medications with fewer side effects is universally acknowledged.

Many of the medications currently available, such as lithium and chlorpromazine, were discovered through serendipitous observation. However, there are more rational and reliable approaches to drug discovery. One approach is to identify genes and proteins thought to be etiologically involved in the disease and then identify the molecular pathways associated with these genes and proteins. Once putative disease pathways or mechanisms have been identified, chemical and molecular libraries can be screened for effective compounds. This approach may also identify molecular pathways impacted by existing medications used to treat other diseases. This approach, called "repurposing," is evident in current trials, such as one using a gout drug, allopurinol, to treat schizophrenia. Another approach is to develop animal models of symptoms, such as the rodent-forced swim test for depression, and then identify compounds that improve it. Finally, one can identify environmental factors thought to interact with the predisposing genes, such as an infectious agent, and treat these factors.

"Gene finding" has been at the forefront of recent psychiatric research efforts and has demanded larger and larger sample sizes and ever more sophisticated computing tools. However, there is an increasing need to integrate the genetic data with the neurobiology and systems biology to achieve improved and more rapid advances in treatment options. We believe that a promising route to drug discovery is to start with postmortem brain tissue from individuals affected with these diseases and then integrate neuropathological, proteomic, neurochemical, and genome-wide expression data on the same set of brains to identify disease-related pathways. We have set up such an online database, the Stanley Neuropathology Consortium Integrative Database (SNCID; http://sncid.stanleyresearch.org), which is freely available to anyone to use for the identification of novel targets and the verification of existing targets. The database currently integrates 1749 datasets from neuropathology studies using 12 different brain regions and genome-wide expression microarray datasets from 3 independent studies using frontal cortex and cerebellum. All data are derived from the same 60 case subjects, 15 each with schizophrenia, bipolar disorder, and severe depression and unaffected control subjects.[1] We plan to add single nucleotide polymorphism (SNP) array data, microRNA array data, and methylation array data from the same cohort and eventually to add similar data on another cohort of 105 brains, 35 each with schizophrenia and bipolar disorder and unaffected control subjects. User-friendly statistical tools such as descriptive analysis, variance analysis, and correlation analysis are included. An interface links the SNCID to a functional annotation database, DAVID (http://david.abcc.ncifcrf.gov), that enables users to efficiently explore genes and pathways that underlie schizophrenia and that may be potential molecular targets for therapeutic drug development. Our initial study using the integrated database demonstrated several potential applications that could potentially lead to drug target identification.[2] We identified several genes whose expression levels were significantly correlated with neurotransmitter levels in different brain regions of the 3 psychiatric disorders and unaffected control subjects. The genes were associated with various biological pathways that could be further explored as potential drug targets.

Studies of postmortem brain tissue have already demonstrated their usefulness in drug discovery. For example, abnormalities of the γ-aminobutyric acid–mediated (GABAergic) interneurons and glial cells have consistently been identified in brain samples from patients with schizophrenia in the Stanley Medical Research Institute and other brain cohorts and by many independent researchers (for reviews, see Lewis et al[3] and Höistad et al[4]). Based on an extensive investigation of the neuropathology of the GABAergic neurons in schizophrenia and follow-up studies utilizing various molecular and

[1]To whom correspondence should be addressed; tel: 240-499-1186, fax: 301-251-8602, e-mail: kims@stanleyresearch.org

physiological strategies, Lewis et al[5] at the University of Pittsburgh have now begun testing a potential medication that acts on a specific GABA receptor. The preliminary findings indicate that the medication improves behavioral and cognitive functions that are not treated by traditional medications. This is an example of rational drug discovery that evolved from initial observations on abnormalities of the GABAergic interneurons in postmortem samples from the frontal cortex of patients with schizophrenia.

Another example of drug discovery derived from neuropathological information comes from the suggestion that oxidative stress–induced neuronal cell death may be the potential mechanism that underlies cytoarchitectural abnormalities such as reduced neuronal and glial density in schizophrenia.[6] Glutathione is the main cellular antioxidant that protects cells from damage by reactive oxygen species, and glutathione levels are decreased in the postmortem brains of patients with schizophrenia. A recent clinical study examined the therapeutic effects of *N*-acetyl cysteine, a glutathione precursor molecule, in schizophrenia and found improvement of negative symptoms that are again not normally improved by the current antipsychotics.[7,8] This is another example of how a translational study from observations at the cellular level in postmortem samples can eventually lead to drug development for schizophrenia.

An additional approach that has potential for discovering novel drug targets for schizophrenia is to use RNA expression profiling of postmortem brain tissue by advanced genome-wide microarray technology (for review, see Altar et al[9]). Since the first successful initial trials with postmortem brain tissue,[10,11] numerous studies using gene expression microarrays have compared RNA levels in postmortem brain tissue from patients with schizophrenia and unaffected control subjects. The studies have yielded many potential new molecular targets for drug development. RNA levels of genes associated with oligodendrocyte/myelination, mitochondria/energy metabolism, ubiquitination/proteasome pathways, immune response, and programmed cell death were significantly and consistently changed in the prefrontal cortex of schizophrenia patients compared with unaffected control subjects. The influence of the current antipsychotic drugs on the expression of some of the genes also increases the possibility of this approach for novel target identification.

The question may arise, however, as to why an expression profiling study should be done with postmortem brain tissue rather than on more readily available samples, such as blood lymphocytes or animal models. The most obvious answer is that the expression patterns of genes in brain tissue are significantly different from those in other tissues,[12] and a significant number of transcripts are brain specific.[13] Moreover, while rodent models may be essential at certain stages of drug development, there are limits to using rodent brains for initial target identification because many candidate genes or splice variants may not be expressed in the rodent brain or may be absent from the rodent genome. An example of such a gene comes from a recent study that identified a primate- and brain-specific isoform of a potassium channel gene, *KCNH2-3.1*, as a risk gene for schizophrenia as well as a novel target for drug development.[14]

RNA expression levels can also be used as a quantitative trait in genome-wide association studies to identify the genetic causes that contribute to the abnormal gene expression found in the disorder. Understanding the genetics that underlie the gene expression changes in schizophrenia may lead to molecular targets for drug development. Recent association studies have shown that DNA variation, eg, SNPs, plays a significant role in the regulation of gene expression in humans (for review, see Nica and Dermitzakis[15]). It will be crucial, however, to use expression profiles from brain tissue from people who actually suffered from schizophrenia when identifying candidate gene causalities from expression SNP association analysis. For example, a recent study showed that obesity-related clinical traits were associated with more gene expression profiles in adipose tissue than with expression profiles in blood cells.[16] In addition, there were more expression profiles detected with significant *cis* expression SNPs in the adipose tissue than in the blood cells.[16] Another study conducted identical genome-wide screens in blood cells and brain tissue and identified polymorphisms with clear effects on both gene expression and splicing; however, the effects were tissue specific.[17] Thus, it appears critical that the genetics of gene expression be studied in cells that represent, and are most relevant to, the disease state. Consequently, RNA expression profiles derived from postmortem brain tissue are likely to be the most relevant for this approach.

As genetic, RNA expression, proteomic, and neuropathology data continue to accumulate on schizophrenia, researchers will be able to take an increasingly integrative approach to combine multiple datasets from multiple sources. Such an integrative approach using genome-wide expression profiles to analyze genes associated with cytoarchitectural/neuroanatomical abnormalities may provide molecular targets for drug development. A recent study we carried out showed that the expression level of genes in biological processes such as cellular metabolism, vesicle mediated secretion, central nervous system development, and programmed cell death was significantly associated with the density of calbindin-containing GABAergic interneurons and number of perineuronal oligodendrocytes in the prefrontal cortex of subjects with major mental disorders.[18] In addition, several candidate genes for schizophrenia previously identified from genetic studies were found to be significantly correlated with the cytoarchitectural abnormalities.[18]

Thus, this integrative approach appears to validate the genetic and cytoarchitectural findings as well as add potential genetic causality to the cytoarchitectural abnormalities. Following up on these associations will result in potential molecular targets for therapeutic drug development.

Of course, there are limitations to using postmortem brain tissue for identifying molecular targets for drug development. A pure disease effect on neuropathologic traits is difficult to identify because of various confounding factors. The expression levels of many genes and some cytoarchitectural traits are affected by confounding variables such as brain pH, postmortem interval, age, antipsychotic or other drugs, and other sources of artifact, such as smoking, substance abuse, and metabolic syndrome. Moreover, the effect size of the disorder on gene expression and on most neuropathology traits is small or moderate. Therefore, a large sample size is generally required to detect a significant effect. However, it is usually difficult to achieve a large sample size because of limited postmortem brain availability. Fortunately, solutions are emerging that will help overcome the limitations. For example, sample size for postmortem studies can be increased by organizing nationwide or international brain bank consortiums. While this effort may require significantly more organization and budget and may involve issues such as standardization of anatomy, storage, processing, and ethics, the result will nevertheless provide invaluable resources for neurologic and psychiatric researchers.

The use of brain tissue for drug discovery is likely to become even more important in the future. Technology is likely to continue to improve for the assessment of neuropathological, cytoarchitectural, neurochemical, genomic, proteomic, and metabolomic aspects of brain function, and these measures will become increasingly useful for identifying targets for drug intervention. It is therefore important to carefully preserve brain tissue from psychiatric patients so that it can be used in the future.

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