4th Annual Report

January 2011

CONFIDENTIAL
EXECUTIVE SUMMARY
Director: Ed Scolnick, M.D.

1. OVERVIEW

This report covers the period of January 2010 through December 2010 and includes progress in bipolar disorder genetics, schizophrenia genetics, emerging gene studies, cell models and clinical translational studies. This is the fourth year of the Stanley Center for Psychiatric Research at the Broad Institute. By far, this has been the best year for the Center as judged by

1. Progress made in understanding the underlying genetics of schizophrenia and bipolar illness;
2. Progress in moving from the emerging genetics to neurobiology to understand the underlying biology of the genetic signals;
3. Steady progress in producing iPS cells and neural progenitors from patient fibroblasts. This is setting the stage for the use of this technology in studying the neurobiological impact of malfunctioning genes;
4. Development of chemical screens for novel compounds in novel pathways. Hits from these screens will be used as probes towards possible new treatments in the future;
5. In-depth progress on the chemistry and possible medical applications of HDAC2 inhibitors. This project has reached the stage where we are trying to raise capital to develop the compound as a possible treatment for a variety of cognitive dysfunction in humans, including Alzheimer’s disease, PTSD, and the cognitive dysfunction associated with schizophrenia. We began this project three years ago. For a Center based in an academic institution, achieving such a possible treatment milestone in only three years proves that the Center can excel at using innovative basic research to discover possible new treatments.
6. The beginning of gene sequencing to identify coding mutations in genes that confer risk for schizophrenia and bipolar illness.

The sections in the annual report follow this summary. We have tried to organize the report so that key projects utilizing different disciplines are presented as integrated projects discipline-specific studies are described under each individual discipline (Neurobiology, Chemical Biology, Behavior, Medicinal Chemistry). This executive summary will try to articulate the importance of certain findings both within the context of work done in the Center and other discoveries that have been made in the past year by external investigators, while clearly pointing out what is not the direct work of the Stanley Center. Thus I have combined some of the detailed project, program and appendix sections of the full report in this Executive Summary to highlight these key topics:

- Genetics
- Emerging Genes
- Chemical Screens
- HDAC2 inhibitors as Therapeutics
- Translational Efforts

Lastly, we include an Operational Overview, which describes leadership changes to our organizational structure, and new members of the Broad and MIT community who will
strengthen and expand research in the psychiatric disease field. Additional basic operational information is included.

2. BIPOLAR DISORDER (BPD) AND SCHIZOPHRENIA (SCZ) GENETICS

The Psychiatric GWAS Consortium (PGC) is an international effort dedicated to conducting a rigorous set of meta-analyses on primary genome-wide association studies (GWAS) from five major psychiatric illnesses (autism, ADHD, bipolar illness, major depression, and schizophrenia), to search for definitive genetic risk loci within and across these diseases. The PGC effort, described in past SC reports by Dr. Sklar (and detailed beautifully at https://pgc.unc.edu/index.php; recently published in Sullivan et al. Neuron 2010), was founded less than 4 years ago, initially by the four Principal Investigators of the GAIN (Genetic Association Information Network) study. Today there are over 100 members involved in focused working groups, several of which are led by members of the Stanley Center. The PGC completed a meta-analysis of GWAS studies in schizophrenia, bipolar illness and a cross-disorder analysis of these two illnesses with major depression. The results indicate unambiguous statistical evidence for several loci as risk genes for the various illnesses.

**Schizophrenia**

Highlights of the PGC meta-analysis of schizophrenia include:

1. Association with the MHC locus and the many genes in this region. Because of the high degree of linkage between the many genes in this region it is difficult to discern which gene(s) within this region are the critical genes. Genes involved in chromatin function, genes involved in immune function, and genes involved with synaptic connections are located in this large region. In collaboration with Patrick Sullivan of UNC, a genotyping plan is in place for finer mapping of this region to try to narrow the signal and gain more insight into the biological significance.

2. A micro RNA called miR-137 on chromosome 1 is a very significant finding in the meta-analysis. This micro RNA has been shown to regulate neural precursor growth and differentiation. Among the genes which it is reported to regulate, several are also in the top of the statistical analyses of genes that confer risk for schizophrenia. Thus the discovery of the role of this micro RNA in the etiology of schizophrenia has opened a clear biological direction for beginning to understand the developmental pathways involved in schizophrenia pathogenesis. Dr. Tsai’s laboratory is actively pursuing this observation to discover the underlying biology involved.

3. Clear statistical significance is also noted for ANK3, previously implicated in bipolar illness risk, TCF4, a transcription factor involved in brain development, the WNT signaling pathway and the calcium channel genes CACNA1C, CACNA1D and CACNB3. CACNA1C, an L type voltage gated calcium channel, was previously implicated in bipolar illness. *Importantly, the signals within the CACNA1C gene that point to risk for bipolar illness are independent of the signals in the gene that point to risk for schizophrenia*. A possible implication of this observation is that different variants of the same gene can confer risk for what are classified clinically as two different illnesses. Such allelic heterogeneity has ample precedent in many other medical illnesses (Zoghbi & Warren, Neuron, 2010).
4. A study examining for copy number variants in 600+ Bulgarian trios (2 parents and an ill child with schizophrenia) has detected about 5% of the cases with de novo copy number variants in several chromosomal loci. This data indicated that 5% (or perhaps more from other literature) of schizophrenia is caused by such de novo CNVs. Very importantly, the distribution of genes within these CNVs is not random with respect to all human genes. The CNVs contain a greater proportion of genes that function in the post synaptic density in neurons. This is a very important observation with regards to the abnormal brain function in schizophrenia. It is noteworthy that other work in autism human genetics, a mouse model created by Dr. Guoping Feng (MIT’s McGovern Institute) of obsessive compulsive disorder (OCD) (Welch et al, Nature, 2007) and other unpublished work by Dr. Feng also implicate genes functioning in the post synaptic density. The association with CPG2 noted in bipolar illness also strengthens this hypothesis since CPG2 also functions postsynaptically (Cottrell et al, Neuron, 2004).

5. From earlier work on CNVs and work on the function of the DISC1 gene by Drs. Tsai, Haggarty and Madison, the wnt signaling pathway has also been implicated. Thus in the past 2 years evidence is now available for the wnt signaling pathway, the genes controlled by miR-137, and genes functioning in the post synaptic density. It is clear there are exciting biological directions to pursue. For the first time we are beginning to understand the pathogenesis of schizophrenia, and most importantly, where and how to research to elucidate it. Although the genetic approaches are still at an early stage I am confident that in time major insights will continue to emerge.

6. Remaining doubts about the validity of the polygene score have been eliminated by studies of its transmission in the Bulgarian trios as noted in the Genetics section detailed report. We previously identified large sets of alleles that were, in aggregate, significantly enriched in frequency in cases with schizophrenia compared to matched population-controls. Here we further demonstrate that this set of alleles, identified in an independent population, are on average preferentially transmitted from parents who carry both a "risk" and "non-risk" allele to offspring with schizophrenia. The results of this analysis indicate that the prior evidence for a highly polygenic basis of risk in schizophrenia does not arise from population stratification issues in previous studies.

**Bipolar Illness**

The meta-analysis of the samples from this group of patients has reconfirmed the importance of four genes: (1) The CACNA1C voltage-gated calcium channel; (2) ANK3; (3) ODZ, a gene homologous to a Drosophila brain patterning gene; and (4) a gene called CPG2 which is encoded within a larger gene called SYNE1. CPG2 has been shown to be involved in postsynaptic glutamate receptor recycling by Dr Nedivi and her coworkers (Cottrell et al, Neuron, 2004). The biological significance of CPG2 is note above. An important conclusion from the polygene work and the specific gene associations is that some risk loci confer risk for both schizophrenia and bipolar illness.

Similarities and Differences between SCZ and BP illness: Although the data indicate shared loci between schizophrenia and bipolar illness, an important distinction thus far between these two diseases is the absence of detectable large CNVs in patients with bipolar illness.
Cross-Disease Analysis

A meta-analysis that encompasses major depression has also been performed. A small number of genes achieve statistical significance when all three illnesses are analyzed together. Among them are the aforementioned voltage gated calcium channel and ZNF804A, a transcription factor previously implicated in schizophrenia. Thus several lines of evidence indicate the following hypothesis: SOME FORMS OF SEVERE MENTAL ILLNESS WILL BE CAUSED BY DIFFERENT VARIANTS OF SOME OF THE SAME GENES. This hypothesis has been proven true in many other medical illnesses caused by genetic variation. The implications for the future of diagnostic categories in psychiatry are profound. It seems clear that simple descriptive clinical symptoms will no longer be sufficient for accurate diagnosis once a full understanding of the underlying genetic variation is understood.

Exome sequencing of various genes based upon GWAS studies has also begun. As of the writing of the annual report, the data from these studies is incomplete. At the SAB meeting on January 20, 2011 an update of this work will be presented. The SC plans for genotyping and increased sequencing will also be presented at this SAB meeting. It is apparent, even at this point, that (1) very large scale exome and whole genome sequencing will be needed; and (2) that detailed analyses based upon other available genotyping data and data that can be imputed from the 1000 Genomes Project and other human exome sequencing will be needed. The combination of these approaches will be needed to continue the progress in genetics. Plans for doing such work will be discussed at the SAB meeting.

In summary, three important biological pathways have now been implicated in the pathogenesis of schizophrenia and to a lesser extent in bipolar illness. This is already spawning the development of new screens for new treatment approaches (as described below). CNVs seem to be more diagnostic of schizophrenia than bipolar illness. Schizophrenia and bipolar illness share some genes that confer risk. Schizophrenia, bipolar illness and major depression share some genes that confer risk, leading to the hypothesis that severe mental illness will eventually be diagnosed with the aid of genetic tests and not just clinical symptoms. Major new directions for understanding the neurobiological pathogenesis of these illnesses has already emerged, even at this early stage of the project. A major effort in exome and whole genome sequencing will be needed in the coming years. I am actively involved with other efforts to encourage expanded national support for such an endeavor.

3. EMERGING GENES

Significant beginnings have been made towards moving from the genetic findings in the association studies, including the discovery of CNVs, towards eventually gaining a detailed understanding of the pathobiology revealed by these genetics findings. Important limitations towards that goal still exist. For the CNVs, since multiple genes are deleted, we still are not certain which genes (and whether one or more than one) are contributing to enhanced risk of schizophrenia. For the genes discovered in the GWAS meta-analyses, we still lack exact knowledge of the ‘causal variant’: which nucleotide variation confers the risk and how that variant of the associated gene functions differently. Nevertheless, while work is beginning to determine the exact causal variant, a number of neurobiological and chemical biological efforts have begun to lay the groundwork for future full understanding.
Mouse Models:

As described recently (Nestler & Hyman, *Nature Neurosci* 2010), animal models for psychiatric disorders are challenging to create but could have great benefit to advance progress in understanding pathophysiology and treatment development. For example, a number of mouse lines carrying different mutations in DISC1 have been created in the past 5 years and have exhibited various behavioral abnormalities. In the SC, there are two ongoing efforts to build improved animal models, based on the emerging genetic data.

1. The Behavioral Neurogenetics group has found that mice haploinsufficient for Ank3 exon 1b (which is present only in the brain-specific Ank3 isoforms) display risk-taking behavior in several behavioral paradigms (detailed in the Emerging Genes Project section). This finding is supported by a similar phenotype in mice expressing a short hairpin RNA (shRNA) that reduces Ank3 expression in hippocampus dentate gyrus. The risk-taking behavior is ameliorated by lithium treatment. Thus this model will be further explored as a potential model for the mania phenotype of bipolar illness. In addition the Tsai lab is studying the role of Ank3 in WNT signaling in vivo.

2. The Tsai lab is working to create mice carrying gene deletions analogous to those found in the human CNVs from chromosomes 15q13.3 and 1q21.1. Zinc finger nuclease technology is being applied to this project and a collaboration with Dr. Alea Mills of Cold Spring Harbor Laboratory (CSHL) is also a part of this effort since CSHL has created such a mouse for the 16p11.2 deletion that confers risk for autism.

3. We are collaborating with Dr. Uwe Rudolph of McLean Hospital to create mouse models for the schizo-affective/bipolar-related duplication/triplication of chromosome 9p24.1. This CNV (spanning 1.8Mb in humans, with 1.3 Mb duplicated and 0.5 Mb triplicated) was discovered by Deborah Levy (McLean Hospital) and Jonathan Sebat (then at CSHL) from a family study. This CNV region encompasses 15 genes, all of which have mouse homologs: 14 genes are on mouse chromosome 19, which is largely syntenic to human chromosome 9, while 1 gene (KDM4C, located in the triplicated region) has a mouse homolog on on mouse chromosome 4. Both individual and combined models will be made.

Longer term, several mouse models will be created based upon additional human genetic findings so the field will have mouse models based upon the actual genetic bases of schizophrenia and bipolar illness. Just as such specific models have revolutionized animal testing for drugs to treat human cancers, it is expected they will have the same impact upon finding new treatments for severe mental illness.

Human Neuronal Cells in Culture from Patients with Defined Risk Genotypes:

Two technologies are being developed to enable studies of cells with defined risk genotypes. A collection of fibroblasts is being accrued from patients with various risk haplotypes defined by the association studies and also from patients with specific CNVs. Details can be found in the Emerging Genes section 2C2 (page 34). The Haggarty/Madison group has made good progress in converting human fibroblasts to induced pluripotent stem (IPS) cells and then further differentiating these to neural precursors. The Tsai lab has made progress in converting human fibroblasts directly to neuronal cells (induced neurons or 'iN'). These two complementary methods will be further developed in the coming year. Even as this progresses, a number of exploratory studies are underway to try to relate changes in neuronal differentiation or function to the underlying genetic haplotypes in the cells. The cell lines being
collected for reprogramming are listed below. As many aspects of the SC are, this is also an international cooperative effort.

**Table 1. Cell lines identified and prioritized for reprogramming**

<table>
<thead>
<tr>
<th>Gene/Locus</th>
<th>Rational</th>
<th>Sources</th>
<th>Cell Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>22q11</td>
<td>Highest odds ratio for psych. genetics locus, microRNA connection, PIK4c</td>
<td>multiple sources</td>
<td>Yes</td>
</tr>
<tr>
<td>1q21</td>
<td>Rare CNV, high odds ratio, Wnt connection</td>
<td>Same as 22q11</td>
<td>Yes</td>
</tr>
<tr>
<td>15q13</td>
<td>Rare CNV high odds ratio, Wnt connection</td>
<td>Same as 22q11</td>
<td>Yes</td>
</tr>
<tr>
<td>ANK3</td>
<td>Best BP single locus</td>
<td>Perlis, McLean</td>
<td>No but Individual identified</td>
</tr>
<tr>
<td>CACNA1c</td>
<td>Strong Scz and BP locus, with existing biological assays</td>
<td>Perlis, McLean, Coriell</td>
<td>Yes, reprogrammed</td>
</tr>
<tr>
<td>TCF4</td>
<td>Strong Scz association, 2 independent signals, potential connection to mir-137</td>
<td>McLean, Freedom Trail</td>
<td>no</td>
</tr>
<tr>
<td>SYNE1/CPG2</td>
<td>Top BP candidate gene with strong existing biological assays</td>
<td>Perlis, McLean</td>
<td>Yes</td>
</tr>
<tr>
<td>mir-137</td>
<td>microRNA connection, significant Scz locus</td>
<td>McLean, Freedom Trail</td>
<td>no</td>
</tr>
<tr>
<td>ODZ4/mir-708</td>
<td>microRNA connection</td>
<td>Perlis, McLean</td>
<td>no</td>
</tr>
<tr>
<td>HLA</td>
<td>Among most significant Scz loci</td>
<td>McLean</td>
<td>yes</td>
</tr>
<tr>
<td>DISC1</td>
<td>Psych disease association. Regulator of Wnt signaling.</td>
<td>McLean, Coriell</td>
<td>Yes, 1 variant reprogrammed</td>
</tr>
</tbody>
</table>

Assessment of genes within CNVs

As noted above, although informatic analyses can suggest which genes among those included in the various CNV deletions are important for disease risk, experimental biology is needed to try to provide additional evidence and potentially validate these suggestions. The Haggarty Group in collaboration with the RNAi platform at the Broad have developed a mouse neuronal culture system and imaging measurements of various neuronal functions to study this. By using shRNAs to knock down expression systematically of the genes found missing in the human CNVs, they are trying to define which genes affect neuronal function. Details can be found in the Emerging Genes section. Such studies will help prioritize which genes to study further and which genes to select for a greater sequencing effort.

Other Neurobiology

The Tsai lab, the Haggarty lab, and the Nedivi lab (at MIT) are working on specific genes detected in the human genetic studies to elucidate the malfunction of the risk haplotype. This work is currently focused on, respectively, miR-137 (a microRNA risk gene for schizophrenia), TCF4 (a transcription factor risk gene for schizophrenia and cross-disorder risk gene), and CPG2 (a risk gene for bipolar illness). In addition specific genes deleted in the chromosome 1
and 15 CNVs are being investigated. Details can be found in several sections of this report: Emerging Genes Project Team, Chemical Neurobiology (Haggarty) Program, and the Neurobiology (Tsai) Program Reports.

Molecular Neurobiology

Significant effort is underway to define brain-specific splice variants (isoforms) of the various risk genes. It is expected that knowing these will aid the effort to relate specific nucleotide differences in risk haplotypes to malfunction. The work is focused mostly on ANK3, the L-type voltage gated calcium channel (a multi-subunit structure which contains multiple independently identified risk genes), and the TCF4 transcription factor. Details can be found in several sections of this report: Emerging Genes Project Team Report.

Chemical Biology approaches

It is clear that having a selective small molecule that binds to a given protein and affects its function can be an aid to unraveling the function of the protein. For this reason, a common term for such a molecule is a ‘probe’ (as in the NIH’s ‘Molecular Libraries Probe Production Centers (MLPCN)’). To this end the Haggarty and Madison groups are working with the Broad CB/NT group to screen for small molecules that bind to Ank3. Similar efforts, supported by the PsychHTS Initiative, are underway for CPG2, in collaboration with Dr. Elly Nedivi of MIT and for NPAS4, a regulator of inhibitory synapse development, in collaboration with Dr. Michael Greenberg of Harvard Medical School. The ANK3 project is detailed in the Emerging Genes section, while the other projects are summarized in the PsychHTS Report.

It is important to note that these efforts are occurring in parallel with efforts to develop specific new screens for probes towards new possible treatments (see the next section below in this executive summary). Thus, even while the SC is working on further elucidating the human genetics of schizophrenia and bipolar illness, key early translational efforts are underway.

4. CHEMICAL SCREENS

Four to five large pharmaceutical companies have closed their research on new medicines for severe mental illness. The timing of this risk-averse attitude could not have come at a worse time, as just now the genetics is finally starting to reveal clear pathways and targets to pursue for novel mechanisms of therapeutics. This exit from research means there is an even smaller amount of chemical screening being done, essential for new mechanisms of action and for establishing a robust pipeline for drug discovery. Thus we are exploring novel ideas in the hope of eventually garnering enough information to stimulate the industry to re-enter the field.

The Stanley Center has organized two mechanisms for new screens in the quest to uncover possible novel approaches to the treatment of severe mental illness: projects initiated and performed by SC members, and external projects operated by the ‘PsychHTS’ effort (also supported by SMRI—detailed in the PsychHTS Report). Both efforts are enabled by efforts from the SC Chemical Neurobiology Group, managed by Dr Haggarty, the Chemical Biology and Novel Therapeutics (CB/NT) Group of the Broad Institute, headed by Michelle Palmer, and the SC Medicinal Chemistry Group, headed by Ed Holson. The evolving screens derive from three sources: 1) Ideas generated by Dr Haggarty’s research; 2) Ideas culled from the literature based upon discoveries of any investigator; and 3) Ideas generated from emerging genetic discoveries. In the case of ideas emanating from outside investigators, if the investigator wishes to work actively with the SC and CB/NT, then we fund that work from our PsychHTS pilot fund or we facilitate the outside Investigator’s ability to apply for NIH funds from the MLPCN Program. If we
need to develop the screen fully in-house without significant external collaboration, then we fund it either through the annual SC funds, other gifts to Dr. Scolnick, or from an MLPCN grant for which we apply.

In the past year we have recognized the need to have more support within CB/NT for the PsychHTS screen development in collaboration with outside investigators. Thus new personnel have been added to the CB/NT group to facilitate screen development. The list of screens under development and that have been executed is summarized below. More detail on each screen can be found in either the Emerging Genes Report (for ANK3), the WNT Signaling Report (for GSK3β), the Chemical Neurobiology Report (for TNIK and LSD1), or the PsychHTS Report (for all other screens)

1. Kappa Opioid Receptor Antagonists. This screen originated from a proposal from McLean Hospital. A calcium imaging assay was developed and screen run. Hits are being evaluated by the McLean group. The rationale is for possible treatment of bipolar depression.

2. GSK3β. Jen Pan & Steve Haggarty were awarded an R03 from the NIH’s MLPCN program, which supported a primary screen of 300,000 compounds, and some secondary screening and subsequent characterization. The large screen was designed to search for allosteric inhibitors of this enzyme. The details of the screen are in section 4D of the WNT Signaling Report (page 76). One structural class of chemicals was found but it is known from the literature and no allosteric inhibitors have been found. Excellent structural biology has been carried out with an outside company and a 3.0 angstrom structure of the enzyme bound to the Chiron GSK3β inhibitor has been deduced. The structure has led to novel ideas about future chemical directions and a new approach to searching for chemical allosteric inhibitors—specific for neurons-- is being developed based upon how known peptides in the WNT pathway inhibit this enzyme. The SC believes that a highly potent and specific inhibitor of this enzyme might be a novel therapeutic for both schizophrenia and bipolar illness. We will spend an additional year determining if we can find a tractable chemical approach.

3. Arrestin-Mediated Dopamine Receptor Blocker. Based upon work published by Marc Caron we have developed screens to search for a novel structure that would block selectively the dopamine D2 receptor and its signaling solely via the arrestin intracellular pathway without affecting the D2-mediated cyclic AMP pathway. The known literature surrounding Clozaril and how it signals at D2 receptors suggests the hypothesis that such a compound might mimic Clozaril in effectiveness without the side effects. If such a chemical can be found and designed by medicinal chemistry, we will test it for antipsychotic effects in animal models standardly used to find antipsychotic compounds. We wish to find a totally novel chemotype to begin such an effort since all existing structures that block D2 receptors also interact with a multitude of 7 transmembrane G protein linked receptors (GPCRs). The next year will provide enough information to determine if this project is feasible.

4. FGF22 Binders. This project derives from the recently published work of Dr Hisashi Umemori at the University of Michigan (Terauchi et. al., Nature, 2010). Dr Umemori discovered the differential effects of FGF22 vs. FGF7 on excitatory vs. inhibitory synapses. In consultation with him, he pointed out that the N-terminal domains of these two members of the FGF family are unique to each family member. Thus we have worked with him to develop a screen for small molecules that would selectively bind each FGF. The thermal stability shift assay has been robustly developed by CB/NT and the screen is about to
begin. The concept behind this would be the ability to selectively modulate the activity of each of FGF 22 and FGF7 and to test such binders for possible therapeutic effects in various animal models of behavior.

5. Small Molecule Modulators of GADD45B and FRP3. This screen, with Dr Hongjun Song from Johns Hopkins, is based upon his work showing the effect of electro-convulsive therapy (ECT) on these two proteins. We are searching, via multiple screens, for small molecules that increase expression of GADD45B or that block the binding of FRP3, an antagonist of WNT signaling. CB/NT has shown that an antagonist of sonic hedgehog, an analogous protein hormone, can be found; we want to determine if such a selective small molecule can be found for FRP. Compounds having the desired activities and specificity would be tested to see if they induced neurogenesis in the brain and whether they had antidepressant effects.

6. Other Developing Screens. Based upon work of Dr. Haggarty’s group we are beginning work to develop screens for inhibitors of
   a. A kinase called TNIK as an approach to modulating the WNT pathway downstream from GSK3β,
   b. A phosphatase called MKP1, recently discovered by Dr. Duman (Yale) as being implicated in depression (Duric et. al., Nat Med, 2010).
   c. LSD1, (‘Lysine-Specific histone Demethylase’), a flavin dependent histone demethylase. Inhibition of this enzyme would provide an independent approach to inhibition of HDAC2.
   d. We will be working with Dr. Guoping Feng to develop a post-synaptic functional assay from his SHANK3 (‘SH3 and multiple ANKyrin repeat domains 3’) knockout mouse vs. wild type mice. Mutations in this gene are associated with autism spectrum disorder. However, since dysfunction of post-synaptic signaling has been implicated in both autism and now schizophrenia, this is an important new direction for chemical screens. The attempt to find small molecules that can work downstream of various genetic defects in the post-synaptic function would potentially lead to novel approaches for treatment.

7. For proteins not yet amenable to specific functional assays, we employ different methods, developed and optimized by the Broad’s CB/NT group: small molecule microarrays or thermal stability shift assays to detect molecules that bind the novel protein target selectively. The focus currently is on ANK3 and CPG2, given their role in the genetics of bipolar illness and schizophrenia. Small molecules that specifically bind these targets could be valuable probes for assessing functional changes of these two proteins.

In summary, as mentioned earlier, full details on each screen can be found in either the Emerging Genes Report (for ANK3), the WNT Signaling Report (for GSK3β), the Chemical Neurobiology Report (for TNIK and LSD1), or the PsychHTS Report (for all other screens). We expect in the next two years that many new ideas for screens will derive from the rapidly emerging genetics. It is clear that we are in a position to try high-risk creative approaches that industry will not attempt.

5. HDAC2 INHIBITORS AS THERAPEUTICS

The Stanley Center has had a collaboration between the Tsai Laboratory, the SC Chemistry group, the SC Haggarty group and the Broad’s CB/NT assay development group led
by Dr. Palmer for over 3 years to discover selective HDAC2 inhibitors and evaluate them as possible therapeutics for various cognitive functions in humans. The basis for the hypothesis that inhabiting HDAC2 will have therapeutic value for human cognitive dysfunction derives from two types of data. First, the human Mendelian disease called Rubinstein-Taybi syndrome is due to lack of function of a histone acetylase. This human defect has been reproduced in mice. Such mice have significant learning defects and these can be corrected by nonselective HDAC inhibitors thus reversing the lack of histone acetylation. Second, work from the Tsai lab has shown the effect of overexpression and knock down of HDAC2 in mice and these results confirm the role of HDAC2 (as opposed to the highly similar protein HDAC1) as a facilitator of learning and memory.

In prior years, the SC has shown that inhibiting HDAC 2 with a chemical called SC-027 at high doses can reverse the learning deficits in a severe model of Alzheimer’s disease in mice created by the Tsai lab. In the past year this work has been extended to show that much lower doses (as little as 0.1mg/kg) have the beneficial effect and that correction of defective synaptic histology is corrected in lock step with the clinical benefit to the mice. Specific histone acetylation marks accompany the effects. Every other day dosing of SC-027 also works well. SC-027 works much better than Aricept, a known approved treatment for human Alzheimer’s disease. SC-027 also works in a mouse model of Rubinstein-Taybi Syndrome. Based upon what is known about the human and animal toxicological study of SC 027 it is hypothesized that this compound is safe enough to conduct a human trial in Alzheimer’s disease. This hypothesis will need to be tested in new animal safety tests and careful escalating dose human trials. We are attempting to raise capital to conduct such studies.

Importantly new structures devised and synthesized by the SC chemistry group have been discovered in the past year and the best of these is undergoing behavioral testing in the Tsai lab in the Alzheimer’s model. SC-027 and the new compounds are not mutagenic with or without metabolic activation of liver cells.

The second exciting direction for the project has emerged in the past year. Fear conditioning models exist in mice, and methods exist to carry out extinction of this fear conditioning. These models are believed to phenocopy human PTSD and the cognitive therapeutic approaches that seem to benefit patients with PTSD (Schurr et al., JAMA, 2010). The experimental approaches to modify fear memories, or the memory of conditioned fear, in mice fall into two categories. The first approach is called ‘extinction’: After fear conditioning, mice can be trained to learn not to react to the cues that had been associated with the fear-inducing shock. This process is called extinction and is a new learning process not a forgetting of the fearful memory. The second approach is called reconsolidation. In this experimental paradigm, mice have a short exposure to the dangerous environment and then are placed back in their home cage. The exposure to the dangerous environment in the absence of being shocked allows the old memory to be modified during its labile time window. Thus, reconsolidation allows a reworking of an old memory and extinction allows a new circuit to form to dominate the fear memory. Importantly, HDAC2 inhibition with SC-027 dramatically enhances both paradigms to improve the physical reaction of the mice. This use of SC-027 only requires 1-2 intermittent doses of the chemical, specifically associated with the extinction training or the reconsolidation exposure. We hypothesize that such intermittent dosing of SC-027 in humans will be safe and will prove to be a powerful adjunct to cognitive therapy for PTSD. Again, we are trying to raise capital to carry out such trials.

In collaboration with Dr. Goff and his colleagues at the MGH we have also discussed clinical trial design to determine if SC-027 will improve cognitive dysfunction in patients with schizophrenia. This project is the first of the Stanley Center that has potential tangible clinical applications.
6. TRANSLATIONAL EFFORTS

The goal of the Stanley Center remains to make discoveries grounded in basic science in the understanding of the pathophysiology of schizophrenia and bipolar illness. As such knowledge is gained, we wish to attempt to translate this knowledge into better diagnostic and treatment approaches for patients. In the fourth year of the Stanley Center a small number of such translational efforts have emerged.

1. HDAC2 inhibition in patients: The project using HDAC2 selective inhibitors has gained sufficient positive results in mice that we are attempting to raise capital to test SC-027 and subsequent compounds in humans for cognitive benefit. Possible clinical conditions include Alzheimer's disease, post-traumatic stress disorder (PTSD) as an adjunct to cognitive therapy, and schizophrenia to improve cognitive dysfunction. Details of this project are in the HDAC2 section of this report.

2. Isradipine in bipolar depression: The SC genetics program has identified the L type voltage-gated calcium channel as a risk gene for both schizophrenia and bipolar illness. The exact functional or nucleotide variation within the channel gene has not yet been identified. Nevertheless, a pilot clinical study has been ongoing, conducted by Dr. Roy Perlis of the MGH Dept. of Psychiatry. He identified isradipine as the most brain-penetrant calcium channel blocker and has been testing it as adjunct treatment for bipolar depression. The study blinding allows preliminary assessment of the results. Thus far in a small number of patients, isradipine is well tolerated at 10 mg per day and seems to have preventive effects on depression recurrence, as described in more detail in the Clinical Program Report. An NIH grant to study a larger cohort is being written.

3. Diminished WNT signaling is postulated to be a factor in human clinical depression and bipolar disorder based upon the basic research conducted by the Tsai laboratory (see page 68). Dr. Haggarty and Dr. Perlis have evidence that statins and lithium synergize to activate Wnt signaling in human cells. They plan a grant to study this combination in humans in bipolar disorder.

4. Biomarkers for bipolar illness: based upon the basic science work implicating the WNT signaling pathway in the pathogenesis of schizophrenia and bipolar illness and the role of AKT in the response to lithium treatment (see GSK3β section 4D, page 76). We have embarked on an exploratory approach to determine if in Lymphocyte Cell Lines (LCLs) derived from patients with bipolar illness we could detect biochemical differences between samples from patients vs. samples from controls. We have not yet done experiments on such samples from patients with schizophrenia. Our two different but complementary approaches are described in more detail in the Wnt Signaling Project Report:
   a. Dr. Jen Pan has presented data (page 75) that suggest LCLs from bipolar patients have a higher amount of phosphor-Akt308 than LCLs from controls. This should be regarded as a preliminary result, until many more variables that could affect pAkt levels can be studied in LCLs. So far, however, it is clear that obvious variables such as growth phase or rate or the days after culturing the cells do not account for the difference.
   b. The Tsai lab has infected such LCLs with a TCF LEF reporter construct as noted on page 69. In a large number of samples from bipolar patients vs. controls, it appears that Wnt 3a stimulated expression of the TCF LEF reporter is substantially lower in LCLs from patients vs. controls. Variables that might confound this result have been
investigated: Wnt 3a concentration, EBV content or growth phase of the cells, the time course of expression, and source of the cells. Preliminary analyses suggest none of these factors account for the difference measured.

There are no data yet on fresh cells, as we have wanted to solidify these results in cell lines before embarking on a new human subjects study to allow testing fresh lymphocytes. However, now that we have a clear hypothesis, we will work with Dr. Roy Perlis at MGH to develop a plan to test blood from patients and controls.

Overall, we are encouraged by this data thus far and will continue to pursue it to determine if these observations could lead to biomarkers for patients with bipolar illness and schizophrenia.

7. OPERATIONS OVERVIEW

Productive focused research is made possible by optimal organizational structure, leaders, and management practices. This section highlights some of these key issues.

As mentioned in the Genetics section, one big change occurring this year is that, effective February 1, 2011, Dr. Sklar is leaving her position at MGH to accept a position as Professor of Psychiatry and Genetics at the Mount Sinai School of Medicine where she will be developing a Division of Genomics within their psychiatry department. It is a fantastic opportunity for her and it will only help expand needed effort on psychiatric disease research. At this juncture it appears that Dr. Purcell will be moving to New York also. Their roles and involvement with the Stanley Center will necessarily shift to collaborators rather than directors. Thus, a new challenge for us as an organization is finding strong leadership to fill their roles, and managing this transition. Several factors help ease this shift: (1) The fact that Drs. Sklar and Purcell are continuing their research on the same projects, and we intend to maintain strong collaborative relationships; and (2) We all remain members of the many consortia that have been formed to make this complex psychiatric disease genetic research so successful: the International Schizophrenia Consortium (ISC), the International Cohort Collection for Bipolar Disorder (ICCBD), and the Psychiatric GWAS Consortium (PGC). Although we anticipate that our individual research efforts will gradually proceed down different paths, we all share the common goal and passion of definitively elucidating the genetic bases of these illnesses.

Moving forward, the Stanley Center Genetics Team will be directed by Dr. Steven McCarroll, with the aid of Dr. Mark Daly. Dr. McCarroll is an Assistant Professor at Harvard Medical School in Genetics (his CV is included in Appendix A). We are quite fortunate that Dr. McCarroll came to the Broad Institute in 2004 as a postdoctoral fellow with Dr. David Altshuler. He has recently made many important contributions in discovery and analysis of copy-number variation and regulatory polymorphisms in the human genome, and relating such variants to risk for complex disease. This experience, combined with his previous training as a neuroscientist (with Dr. Cori Bargmann on C. elegans) and personal interest in psychiatric disease, make him ideally suited for the position. We are excited about Dr. McCarroll joining us. An example of his approach and critical thinking can be seen in his project description in the Emerging Genes section 2B (p.30). Finally, we recognize the time and effort required to assume such a role. The involvement of Drs. Sklar and Purcell, experience of our Genetics Program Manager Dr. Jennifer Moran and Project Manager Kim Chamber, and plans to hire a staff statistical geneticist will provide Dr. McCarroll both the necessary continuity and support.
Another Directorship change this past year is in the Medicinal Chemistry group. Dr. Mike Moyer, who started the group in summer of 2007, left in March 2010 for the opportunity to create and lead a chemistry group at a start-up company. Fortunately, Dr. Moyer had hired several strong scientists into the Stanley Center team: Dr. Ed Holson, who had been the lead scientist on our HDAC2 project, was a natural candidate and was thrilled at the chance. Dr. Holson has been an enthusiastic, effective Director of Stanley Center Medicinal Chemistry since late March.

A new leadership role that we added this past year is Dr. Roy Perlis of MGH as our Director of Clinical Projects. Dr. Perlis (whose CV is also included in Appendix A) has been involved with the Stanley Center since its inception. He is an Associate Professor in Psychiatry at Harvard Medical School and has been affiliated with the Psychiatry Department at MGH for over 10 years. Dr. Perlis serves as the Director of the Bipolar Clinical Program (the largest bipolar disorder clinic in the US) and Director of Pharmacogenomics Research in the Department of Psychiatry at MGH. As he succinctly summarizes in his Report (p. 102), the purpose of the Clinical Project Team is to ensure that Stanley Center investigation remains tightly coupled to clinical applications.

A final key development this past year in personnel is the additions to the Broad/MIT neuroscience community. We have been able to recruit to MIT and the Broad three world-class scientists truly interested in working in this field: Dr Guoping Feng to the McGovern Institute and Broad Senior Associate Member, Dr Myriam Heiman to the Picower Institute and Broad Core Faculty Member and Dr Feng Zhang to the McGovern Institute and Broad Core Faculty Member. Their experience and interests are described further in the Neurobiology Program Report (p. 114). It is worth mentioning that five years ago, the Broad Institute did not even have a Psychiatric Disease Program; now in 2010, three (Drs. Scolnick, Heiman and Zhang) out of 9 Broad Core Faculty Members are focused on neuroscience, all involved primarily with the SC/Psych Disease Program. As stated in the Neurobiology Report, we are excited about the future impact on our group and the field, as these three new distinctive faculty members share a passion to ultimately apply their scientific talents to translational psychiatric research.

In last year’s report, we described the critical roles our governance committees (Executive, Operating and Scientific Advisory Committees) play. This list of members has grown over these four years, though it is unchanged from last year. As we begin our fifth year, we will likely need to address reasonable terms for such appointments, as we have imposed on some members now for over four continuous years.

As evidenced by the structure of this Annual Report, with most emphasis on Project Team Reports, the core functional units of the Stanley Center remain our cross-discipline Project Teams. The Stanley Center has the mission to discover the genes that cause schizophrenia and bipolar illness, understand how these genes go awry, and develop new treatments and diagnostic tests for these illnesses. Accomplishing these goals, which are broad and out of the scope of usual academic science, requires a multidisciplinary culture with close interaction. The project team meetings provide an opportune forum in which such multidisciplinary interactions can occur. Even in traditional academic research, such team meetings, if attended by smart interested persons, can generate ideas which can extend the boundaries of what individual investigators might think about or can affect experimentally. Logistically, we rotate weekly meetings, so that each Project Team meets at least once every two months. These Project Team meetings are the primary means of summarizing and communicating progress, fostering critical analysis, discussing and evaluating results, and establishing focus and key goals for each Project. A Group Leader is responsible for the
ongoing progress and presentation of the work. Discussion and ideas for future direction are encouraged from all, with key guidance provided by SC Management.

Collaborations continue to be a vital means to explore pilot projects in new areas and to pursue discoveries identified by the genetics or neurobiology programs and accelerate our overall research progress. In past years’ annual reports, we have included separate progress reports by each collaborator. However, in this year’s report, because of how integral our collaborations have become, we incorporate our collaborators’ research progress in each of the Project Team reports. In the context of collaboration, we should note we are also keeping ongoing meetings with our colleagues at the Stanley Center at Cold Spring Harbor Laboratory, to share results, ideas and plans. We had a half-day joint meeting here at MIT in April 2009, then a large 2-day Banbury Conference in September 2009, and we are scheduling another 1-day meeting, here at the Broad this spring.

Our Stanley Center Seminar Series also continues to be a key tool for us to explore new ideas, technologies and fields, potential collaborations, and alternative perspectives, all immediately relevant to our Stanley Center mission. We aim for biweekly seminars (for a total of 20-25 each year), though we keep scheduling flexible to accommodate speakers’ availability. The seminar schedules from last year (’09 – ’10) and the current ’10- ’11 academic year (see Appendix B) reflect the breadth and quality of topics and speakers.

In closing, we want to mention an event we are planning for September 2011. The ‘Symposium on the Emerging Genetics & Neurobiology of Severe Mental Illness’ is being jointly organized by myself, Li-Huei Tsai of MIT’s Picower Institute for Learning and Memory and Guoping Feng of MIT’s McGovern Institute. This 2-day symposium, scheduled for September 22-23, 2011, will bring together leading scientists working on emerging genetics of schizophrenia, bipolar disorder, autism and other mental illnesses (see agenda in Appendix C, p. 170). We believe the timing will be ideal, as emerging genetics are beginning to point to new molecular understandings of the pathophysiology of these diseases. We do not anticipate needing SC funds for this event. We are seeking outside support (and have already received 25% of our estimated budget) to eliminate or drastically minimize any registration fee, as we are particularly interested in attracting graduate students and postdoctoral associates to help build the field at this critical time. We are excited about what 2011 will bring.