5th Annual Report

January 2012
EXECUTIVE SUMMARY
Director: Ed Scolnick, M.D.

1. OVERVIEW

The Stanley Center for Psychiatric Research (abbreviated as ‘SC’ hereafter) was founded in February 2007 as a translational research center with the goal of discovering the genetic and molecular causes of schizophrenia and bipolar illness and to translate these discoveries into improved treatments and diagnostic methods. The Center is just completing its 5th year, and I am ecstatic that this year our progress has been the best ever. I recognize I say this every year—and mean it, as we are constantly improving—but particularly at this 5-year milestone, I think our accomplishments and evolution as a Center merit reflection.

Reflections at Five Years

Genetics and Patient Sample Collection Efforts are Key to Attaining Our Goals

First, with respect to our Genetics program, when we began in 2007, we did not yet have any results from the fledgling ‘genome-wide association study’ (GWAS) approach applied to bipolar disorder or schizophrenia. The initial GWAS of each disease revealed one or two significant risk loci, generating some early excitement about the role of ion channels in bipolar disorder and large CNVs (Copy Number Variations—both deletions and duplications) and the MHC (Major Histocompatibility locus) in schizophrenia. We debated when to ‘pull the trigger’ on functional studies—that is, whether to invest significant resources into dissecting potential functional disease mechanisms of a particular gene (such as ANK3) from such preliminary data. Some in the field were ‘naysayers’, decrying the utility of the GWAS approach and saying it was a waste of money. However, we took those early results, coupled with lessons from other complex genetic diseases such as Crohn’s and type II diabetes (also being studied at the Broad), as encouragement that the genetic approach was clearly sound and would indeed lead us to novel underlying disease mechanisms that were essential for meaningful rational drug development. But more power (that is, greater numbers of patient samples) was needed than we had initially anticipated.

Thus, in 2008, we spearheaded a meeting of research and funding leaders in the field to discuss the critical need for increased samples. As a consequence, both SMRI and the NIMH committed a combined $40 million, with $10 million from SMRI (described in the ‘Patient Collection’ report) and $30 million from NIMH, for both schizophrenia and bipolar patient sample collections. In addition, as genetic analysis technologies evolved and allowed even more fine-tuned detection than GWAS chips (though at greater cost), such as whole genome or whole exome sequencing, we have sought to incorporate these as quickly and strategically as possible to reach our goal of discovering genetic and molecular causes of these diseases. By 2009 a key revelation in the field, based on GWAS results, is that autism, and schizophrenia share some risk genes and that schizophrenia and bipolar disorder clearly share some risk genes. Although such notions have been around for at least 5-10 years, this insight evolved to solid facts due to the recent genetics studies. I noted in 2009 that a key milestone for us—and for the entire field—would be to link a particular genetic variant to a biochemical function to the disease biology. Functional pathways have now begun to be identified; however, a specific functional defect in a risk gene has not yet been achieved. I believe we are tangibly closer to this goal as I will discuss below.
Second, in 2007, while it was clear that deciphering the genetic bases of schizophrenia and bipolar illness would require years of intense work, we also recognized that our ultimate goal is to translate the genetic findings to therapeutics. In addition, we appreciated the substantial effort required to establish the teams, experience and culture required for successful drug discovery. Thus we began in parallel (with the genetics efforts) to search for targets that might lead to new therapeutic approaches before the genetics could lead us to new targets for psychiatric disease. Our HDAC and our DISC1 and Lithium (now joined as ‘Wnt Pathway’) project teams arose out of this rationale. The HDAC project succeeded in being our first bona fide drug discovery project (2008), and advanced to the point where we have both unambiguously validated the mechanism of HDAC2 inhibition for enhancing cognition and memory and developed a compound with a strong pre-clinical profile that we strongly believe is ready for clinical trials. Our clinical team has even helped in designing an effective clinical trial. But the next steps are beyond our scope, our budget, and our focus. We have thus made the difficult strategic decision to end our SMRI-supported significant efforts on the HDAC project in 2Q 2012. We are enthusiastic and hopeful about the Broad’s efforts to license out this compound and have it tested in the clinic. This will require a Broad-MIT joint effort since some of the intellectual property associated with this project stems from the Tsai laboratory effort. The field of HDACs may have further potential for broader relevance to psychiatric disease (as suggested by a few ‘hits’ of modest significance in genetic analyses), but at this time, given the landscape of the types and numbers of highly disease-relevant targets being identified by the genetics, we must shift our focus and our resources to other projects.

Foundation Established for Successful Chemical Screens and Medicinal Chemistry Efforts

Similar to our decision to pursue HDAC inhibitors before solid target and functional data emerged from the genetics, we also decided in 2007 at the outset of the SC to form two other project teams. Initially, these projects were viewed as distinct and had different rationales: (1) The ‘Lithium Project’ had the main goal of finding small molecules that either mimic or enhance the therapeutic efficacy of lithium (without the side effects); to meet this aim, significant effort was invested into cell, molecular, and chemical biological approaches to understand the elusive mechanism of lithium’s therapeutic action; (2) the ‘DISC1 project’ which sought to investigate in mouse neurodevelopment the function of this apparent psychiatric risk gene, identified from a single Scottish family almost 10 years ago. Except for the original Scottish family, it is now clear that neither rare nor common variants of the DISC1 gene confer risk for psychiatric illness. Nevertheless, research focused on DISC1 and lithium have converged, after the discovery by SC scientists that the DISC1 protein inhibits GSK3β (Mao et al., (2009) Cell 136:1017), showing the underlying biology appears to be the Wnt signaling pathway. Thus in fall 2010 we merged the two project teams into the ‘Wnt Signaling Project Team’, to focus on multiple proteins involved in this pathway, including DISC1, GSK3β, Akt, and PDE4D9. Our interest has only continued to increase, as genetic analyses (GWAS and exome sequencing) reveal genes encoding proteins in this pathway have specific risk-associated variation or are located in chromosomal regions of CNVs associated with schizophrenia. I highlight several findings below.

Fruits of Our Labors

We are now clearly reaping the benefits of our strategic decisions and investments. As summarized below (and elegantly articulated in the Genetics Report), we are starting to discern clear functional pathways defined by both common and rare risk loci. These discoveries are giving us a handle on which to base specific functional studies that can help us dissect the mechanism(s) of the aberrant function(s), thus eventually leading to specific ideas and assays to discover novel therapeutics. I must note that we could not have done this work as quickly or
effectively anywhere other than the Broad Institute. The Broad’s combination of expertise in developing genetic technologies and the community of population genetics researchers, particularly psychiatric disease, combined with a culture of collaboration is unique.

Personally, I feel the state of this psychiatric research field is reminiscent of the late 70’s, when the discovery of retroviruses and oncogenes such as ras and their mechanisms completely changed the field of cancer research. That ‘heyday’ of research was dizzyingly exciting and fruitful, with breakthrough discoveries occurring yearly. I am pleased and honored to have a second chance at being part of another similarly exhilarating phase of research, in a field that has been so devoid of breakthroughs in decades and that means so much to me.

Organizational Changes

Normally, I would mention organizational issues in my last ‘Operational’ section of my Executive Summary. However, several important organizational events have occurred and have had tremendous lasting impact on our Center, so I highlight them here. Dr. Steve McCarroll, Assistant Professor of Genetics at Harvard Medical School, assumed responsibility for the SC genetics group after Dr. Pamela Sklar moved to Mount Sinai School of Medicine in NYC on February 1, 2011. Our genetics effort has also become more strongly aligned with the parallel effort in genetics of autism and ADHD led by Drs. Mark Daly and Ben Neale of the Broad Institute and Massachusetts General Hospital. (Funding for those programs is independent of the SC funds.) These two organizational changes have greatly strengthened the SC efforts in genetics. Dr. Shaun Purcell and Dr. Sklar remain important collaborators in the program, and Dr. Purcell retains an appointment in Dr. Daly’s Unit (‘ATGU’ or Analytic and Translational Genetics Unit) at the MGH. As you will see in the detailed Genetics Project Summary, the genetics strategic plan has become focused, well articulated, rigorous, and assures us of successfully identifying definitive risk loci.

The other groups remain as they were. Three important additions have been made to our SC Management Committee: (1) Dr. Jennifer Moran, our Genetics Program Manager, joined the SC Management Committee in recognition of her key role and excellent performance. Dr. Moran has had responsibility for coordinating all details of the genetics studies, which includes assuring we have appropriate IRB approvals, managing and tracking the many cohorts in our ever-growing patient DNA collection, selecting and coordinating with the platforms the specific study designs and timing, and coordinating data releases with all analysts and collaborators. (2) Dr. Steve Hyman joined the Broad on a sabbatical from Harvard and has rapidly become an integral member of the SC. (3) Dr. Guoping Feng, recruited over a year ago from Duke University as a Professor at the McGovern Institute of MIT, also became a member of our Management Committee, as one of his key interests in moving here was the Stanley Center work. Now that he has finished closing his Duke lab, he is becoming more involved in the neurobiology program of the SC. Thus, the Stanley Center was greatly strengthened in 2011.

Finally, due to the progress made in the first four years, SMRI generously agreed this past year to extend the SC for another ten years, until 2022. The combination of the strengthened staff and the extension of funding from SMRI have further energized the Stanley Center. The SC scientists are totally dedicated to make breakthrough discoveries that will move treatment options forward and further our understanding of these two illnesses.

Thus, it is with utmost sincerity and satisfaction that I reiterate: the progress in both the genetics research, clinical activities, and new chemical screens has been the best ever in the 5
years of the Center, and I am excited to think of the developments and discoveries we will make in 2012. I believe that by the end of 2012 the face of psychiatric research into severe mental illness will have changed forever.

2. GENETICS

Genome-wide association studies (GWAS) carried out between 2007 and 2010 identified a number of risk genes for schizophrenia and bipolar illness. Given the complexity of the genetics, as we articulated last year, we elected to focus our efforts this year on schizophrenia in order to gain a more in-depth knowledge before delving more deeply into the genetics of bipolar illness. The strategy of the program in 2011 has been to employ three simultaneous approaches to further the genetic understanding of schizophrenia: (1) large scale exome sequencing, (2) continued GWAS; and (3) a new ‘exome chip’ to evaluate the impact of coding variants of ‘rare-ish’ (down to 0.1%) frequency. First, the large-scale whole-exome sequencing has continued via two complementary approaches: in both a population-based case-control cohort (Swedish schizophrenia samples) and a family trio-based study. The population-based study aims to identify exome variants that in aggregate are found more in cases with schizophrenia than in controls. To date, about 1500 samples (750 each cases and controls) have now been whole exome sequenced, and some enticing, though tentative due to inadequate power, discoveries have been made. The trio study (a trio is an ill child and two well parents) allows detecting de novo mutations that occur in the ill child that might contribute to the child’s risk for schizophrenia. To date, about 200 trios have been exome sequenced (about half each at the Broad and at the Sanger Center in the UK). We are grateful to Dr. Patrick Sullivan of University of North Carolina and Dr. Christina Hultman of the Karolinska Institute for their collaborative efforts in the population-based study, and we are equally grateful for the collaborative efforts of the Cardiff investigators in the trio-based study.

Second, additional GWAS studies have been carried out on 2921 schizophrenia cases and 3880 controls from the Swedish collection, adding significantly to the recent published meta-analysis of 9394 cases (SCZ PGC (2011) Nat Gen 43:969). As predicted based on GWAS of other complex genetic diseases (such as Crohn’s disease and Type II diabetes), we are experiencing the ‘sweet spot’ of GWAS analyses and are finding more risk loci of high significance. Third, an exome chip containing all protein-coding variants ascertained in at least three humans (among 11 thousand sequenced in human genetics) was designed by Dr. Ben Neale and his collaborators. This chip is being used to quickly and inexpensively evaluate exome variants segregating in the population to identify any rare—but not ‘private’ (occurring only in a single individual)—variants that are present more frequently in schizophrenia cases than controls. Thus we are now able to evaluate common variants in GWAS, rare (but not private) coding-sequence variants with the exome chip, and private coding-sequence variants with exome sequencing. In another six months or so, we will have at least five times more data than is now available. As we digest this influx of new data, we will make our plans for extending these efforts to the genetics of bipolar illness.

Our genetics community is also innovating on molecular and statistical analysis methods in ways that are laying the groundwork for discovery in the coming years. Dr. Ben Neale’s leadership in designing an "exome array" for human genetics research is enabling a novel direction for our gene discovery strategy. Dr. Steve McCarroll's lab is making experimental advances in allowing gene-regulatory influences to be mapped and quantified in neurons and post mortem brain, and in allowing multi-allelic copy number variation to be analyzed for relationship to phenotypes. Innovation in statistical analysis methods by Dr. Shaun Purcell and
others remains a strength in our program. We expect this work to enable genetic insights in 2012 and beyond.

**Key Genetic Insights**

Even before we have this 5-fold increase in data expected in 2012, major discoveries have been made:

1. The polygene signal previously described (Purcell et al., (2009) *Nature* 460:748) from a schizophrenia population has been further validated. In a study of the transmission of this signal in Bulgarian trios (Kirov et al., (2011) *Mol Psych*), the polygene case variants are preferentially transmitted to the child. This excludes the possibility that some undetected population variation (stratification) accounted for the original findings and refutes that proposed explanation (McClellan & King, (2010) *Cell* 141:210).

2. In that Kirov et al study of trios, 5% of ill children were found to harbor a large *de novo* CNV. With a background rate between 1-2% in available control samples (although comparable trios where the child is not ill have not been analyzed), this confirms the involvement of CNVs in the pathogenesis of schizophrenia.

3. Computational methods have been employed to determine whether any functionally meaningful sets of genes carry an increased ‘burden’ of mutation associated with disease. Specifically, are the risk genes identified either by GWAS, by being present in a CNV region, or by containing exome variants (found in the Swedish cases vs. controls or *de novo* in the Bulgarian trios) randomly distributed among the 20,000 human genes or are they enriched for genes that fall into specific functional categories? Using public annotated databases as the source of functional categories, statistical methods have been employed to determine if genes in the above studies fall into selected categories in statistically robust ways. Remarkably such analyses of all the sources of data point to genes enriched in synapses and synaptic function. Thus the findings do not reflect a random assortment of human genes. Consistent with this finding for schizophrenia and with the observation of some risk genes being shared by multiple psychiatric illnesses, rare Mendelian variants previously detected in autism cases have also implicated synaptic dysfunction.

4. Some of the new genes and functional categories implicated are:
   a. *GRIN2A*, an NMDA receptor subunit
   b. Proteins from the E3 ubiquitin ligase family, which provide the substrate specificity for the ubiquitination of proteins and hence their turnover. An example of dysfunction in one such E3 ligase gene, *UBE3A*, has been discovered previously in a brain developmental disorder and also implicated in autism. I am guessing we will find that synaptic protein turnover may be a functional consequence of dysfunction in these proteins.
   c. Four different subunits of L-type voltage-gated calcium channels
   d. Various pre- and post-synaptic proteins
   e. A microRNA gene, *mir-137*, which regulates expression of many genes, several of which have also been implicated as risk loci; and
   f. Multiple Wnt signaling pathway components, including tentative evidence for the signaling mediator *DVL1*. 
Models of Pathogenesis and Functional Plans

In addition to continuing this discovery effort, work has begun to prove dysfunction of the proteins encoded by the various risk genes. The goal is both to gather molecular evidence to support the genetic findings and to allow assays to be devised for drug screening for molecules that will correct the synaptic (or other) dysfunctions. Dr. Feng and Dr. Hyman are leading our efforts in this exciting new direction. Although just the tip of the iceberg given our expected 5-fold increase influx of data, these preliminary data suggest two working models of the pathways involved in the pathogenesis of schizophrenia. Both can be viewed as working hypotheses, allowing for experiments to be designed to prove or disprove them.

Wnt Signaling Pathway

One model is the Wnt signaling pathway, outlined in Figure 1. DVL1 is noted in pink, immediately downstream of Wnt/ Frizzled. Not only is DVL1 a gene for which exome sequencing identified a variant only in a schizophrenia case, but a mouse knockout of this gene was shown to have behavior and sensorimotor gating abnormalities (Lijam et al., (1997) Cell 90:895). Prior data have also noted several steps in the pathway implicated by both conventional biochemistry (drugs such as rolipram, lithium or antipsychotics) and emerging genetics. Fortunately, our chemical screen development has been and will be focused on this pathway, seeking new therapeutics and this will be summarized below.

![Figure 1. Diagram of Wnt signaling pathway. Genetic loci implicated as risk loci for either schizophrenia and/or bipolar disorder are noted in parentheses.](image-url)
Synaptic Transmission

The second model (diagrammed below and elaborated in the Emerging Genes Project report) attempts to provide a hypotheses that integrates many of the neurobiological findings thus far that have been derived from the neurobiology work already being carried out based upon current genetic understandings. This should also be viewed as a working hypothesis. But because of the strong evidence implicating synaptic dysfunction, we have already begun with Dr. Feng to devise a chemical screening approach to look for molecules that will correct synaptic dysfunction caused by various genes.

As detailed in the Emerging Genes section, he has focused initially on post-synaptic assays using his Shank3 knockout (KO) mouse, a model for autism. Shank 3 is an important post-synaptic scaffolding protein involved in neuronal signaling. Although Shank3 has not been directly implicated in schizophrenia, it is a convenient first model, particularly as we already know from discoveries of CNVs that autism and schizophrenia share some risk genes. Dr. Feng has been able to show for the first time that neuronal cultures taken from the Shank3 mice recapitulate the electrophysiological abnormalities previously detected in living brains from these animals (see Fig. 2).

A high-throughput version of this assay will be developed in 2012 and then chemical screening will begin. As other functional effects are found based upon the other synaptic genes discovered, work will begin constructing mice with the appropriate genetic change and then developing additional screens.

Plans to Functionally Test Models/ Genes/ Pathways Identified by Genetics Specific Proteins

We are planning, with Dr. Hyman’s leadership, how to further our biological knowledge as the gene discovery effort proceeds. He has engaged experts in different neuroscience communities to work with us. We have already established connections with Dr. Wade Harper,
an expert at HMS on the ubiquitin pathway, and Dr. Rob Malenka of Stanford (and formerly on our SAB) and Dr. Richard Huganir of Johns Hopkins (and our SAB) on the synaptic proteins. Dr. Haggarty's and Tsai's labs are working on the variant of Dvl1 to determine if it is functionally abnormal vs. the wild type form of the protein There is an enormous amount of work that still needs to be done both in gathering more genetic data and in determining the biochemical consequences of the genetic changes. However it is clear that we are at a new beginning of our insights into the pathogenesis of this illness.

**Protein-Protein Interaction Networks**

Scientists from Dr. Daly's group have also used computational methods to provide an early model of a possible protein-protein interaction ('PPI') network based upon the early GWAS risk genes discovered in 2010. This model (see Fig. 3 for an example output which highlights miR-137 targets present in schizophrenia risk genes) will be updated as new data emerge. The SC plans to work with members of Dr. David Root's RNAi Platform and Dr. Steven Carr's Proteomics Platform at the Broad Institute to express tagged protein constructs in appropriate cells and a SC scientist to test the interaction models in wet lab experiments. Drs. Hyman and Haggarty are leading these efforts. Translating the protein network model into concrete proven protein interactions will further our biochemical understanding and again provide rich knowledge for new chemical screens.

![Protein-Protein Interaction Network](image)

**Further Patient DNA Sample Collection**

As mentioned earlier, one key element for successful genetic studies is to have adequate sample size, particularly because gene discovery responds more than linearly to sample size once sample size has reached a critical threshold. In addition, samples from unique cohorts, or cohorts from other races/populations are especially useful in fine-mapping risk loci (due to different recombination hotspots and/or rates in different populations). We are pleased that just this past year, we have initiated or expanded collaborations with groups who have already spent years collecting large cohorts. Our ability to engage these other...
investigators is in large part due to the open and focused efforts of Dr. McCarroll over this past year. For example we are tremendously fortunate to have strong collaborative relationships with the Cardiff University group (headed by Drs. Michael O'Donovan, Michael Owen, Nick Craddock and George Kirov), as exemplified by the Bulgarian trios, and other new cohorts as detailed in the Genetics Report.

As noted in the Patient Collection report, the collection of samples from patients with bipolar illness is proceeding well and we expect by 2013 to have 20,000 patient samples and 20,000 controls. As we learn the best approaches from our schizophrenia studies we will apply the best methods to these bipolar samples. We do not yet have trios with bipolar illness. Because the fecundity of patients with bipolar illness is not impaired vs. that in schizophrenia, this may not be as important an approach for this disease.

In addition, as described in the Genetics report (section 8), new collaborators have been identified by Dr. McCarroll and new collections of samples from patients with schizophrenia are accruing. Some samples of patients with African American ancestry also are becoming available (due to the 2008 NIMH request for proposals). These may be especially useful in fine mapping studies because their haplotypes are shorter than the samples from patients of European ancestry. Drs. Bruce Cohen and Dost Ongur from McLean Hospital have collected 10 new schizophrenia trios in the past 6-9 months and have agreed to continue this effort. We estimate that another 100 trios can be collected in collaboration with McLean, providing a tremendous resource for genetics and the functional studies using reprogramming technology described below (and in Emerging Genes section 3A).

3. EMERGING GENES

The Emerging Genes Report captures the significant and arduously detailed work that our collaborators and we have been conducting to follow up the function of about half a dozen individual risk genes/ loci. However, as described above, we anticipate this coming year an influx of many more genes and pathways identified as risk loci by the genetics studies. Thus, as in 2008, we will need even more to consider carefully when to ‘pull the trigger’ on functional studies –that is, whether to invest significant resources into dissecting potential functional disease mechanisms of a particular gene. One means of addressing this is establishing a ‘medium –throughput’ process to validate and accurately identify true in vivo networks and sets of functionally related proteins that could be screened using a single assay. Another philosophy is that we will need to exercise a balance of being both strategic –such as waiting for more definitive (i.e., more than 1 hit and clear risk association) data to pursue any significant functional biology—but also opportunistic –such as pursuing certain intriguing results on genes or pathways with an expert collaborator who is interested or with our own internal scientists if we have established relevant assays. This latter ‘opportunistic’ path will necessarily be guided by discussions (and perhaps some debates) among the Center neurobiologists and geneticists, as each individual will have a different ‘threshold’ for what they consider ‘intriguing enough to pursue’. I look forward to this coming year with these conversations.

Rather than summarize these various ‘single gene’ functional studies here, especially since Dr. Jon Madison did such a fine job at reviewing them in his Emerging Genes report, I wanted to highlight below two different models or approaches for broad utility that we are investing in.
**Cellular Models**

*Potential Uses and Insights*

As noted in the Emerging Genes report, we are attempting to analyze human neuronal cells and their precursors for abnormal functions caused by risk genes identified from genetics. Although this approach cannot unravel all the abnormalities that must occur in brain and brain circuits, it can provide important knowledge impossible to gain from typical human cell lines or mouse models. The findings of impaired growth and neuronal differentiation of the cells of two affected sons from a bipolar illness family (detailed in the Emerging Genes report) is a first step in this direction. It is particularly intriguing that early data suggest an abnormality in the Wnt pathway. Much more work is planned in the coming year to definitively test this mechanism.

Technical progress has also been made in creating neurons directly from patient fibroblast samples and this approach will also be extended in 2012. We want to thank Dr. Jerry Crabtree of Stanford for advice on one of the methods. Various functional analyses are underway.

The other advantage of cellular models is the ability to eventually translate them into chemical screens to look for molecules that correct the abnormal function found.

*Patient Fibroblast Sample Collection*

Just as building a patient DNA sample collection has been critical to advancing our genetic discoveries, we are also establishing a collection of fibroblasts, for potential use in these various cellular models. Dr. Jon Madison is leading this effort, initiating and coordinating the complex collaborations (some involving investigators from over 5 institutions) that are necessary to make this labor-intensive effort (compared to DNA collections) a success. These efforts and a list of the collaborations are in page 39.

*Mouse Models:*

Dr. Petryshen’s group has noted that mice lacking one copy of the ankyrin 3 gene, one of the first risk genes identified by GWAS for human bipolar illness (Ferreira et al, Nat Genet (2008) 40: 1056), have various behavioral abnormalities. On the face of it, many of these are analogous to abnormalities in behavior detected in patients with bipolar illness. We are fully aware that this model cannot yet be labeled a mouse model of bipolar illness, but the work is a start in the direction to create new mouse models based upon human genetic findings. This work is completely described in the manuscript entitled “Reduced expression of the ankyrin 3 bipolar disorder risk gene induces manic- and depressive-like behaviors” (provided as a separate pdf), which was submitted to PNAS for review last month.

In addition, Dr. Uwe Rudolph of McLean Hospital has worked out methods to create CNVs in mice that mimic two of the chromosomal CNVs (1q21.1 and 9p24.1) associated with schizophrenia and BP illness in humans. This work, described further in the Emerging Genes report (section 3B, page 48), has been partially supported by SC funds.

### 4. CHEMICAL EFFORTS (MEDICINAL CHEMISTRY AND SCREEN DEVELOPMENT)

**HDAC inhibitors**

We are now concluding our current efforts to synthesize HDAC2 selective inhibitors to improve cognitive function. Compounds selective for HDAC1 and HDAC2 vs. all other HDAC enzymes have been made. The compounds have good pharmacokinetics (i.e., reasonable half-
lives and they are brain penetrant). We also have discovered a selective HDAC3 inhibitor. The data accrued in this project over the past four years (described in detail in the HDAC Project Report) are consistent with HDAC2 being the key target. With the considerable help of the Broad’s Strategic Alliances and Partnering group, we are currently attempting to license out the compounds so they can proceed towards clinical trials in Alzheimer’s disease and schizophrenia. We will continue a focused effort to find novel allosteric (non-active site) inhibitors completely selective for HDAC2. This effort is in collaboration with Michelle Palmer’s group in the Chemical Biology Platform of the Broad, and has been enabled by our collaboration with Dr. Tsai of the Picower Institute as well as an NIH grant to Dr. Haggarty. However, as described earlier, with the advancing genetic insights, we have made the strategic decision to shift more of our efforts and resources to targets and pathways defined by the Genetics.

**GSK3β**

From past data in the literature and data from the SC, it has been clear that an inhibitor of this enzyme is likely to be a useful drug for bipolar illness and possibly even schizophrenia. However, even if an ideal molecule were available, safety of this mechanism for chronic use would need to be established. A potentially easier clinical direction for this project has emerged and is further described in the Clinical Report. Briefly, in the past 2-3 years various clinical trials have shown that ketamine administered intravenously twice to patients with treatment-resistant depression led to dramatic rapid relief of their symptoms. In the past year, data have been published indicating that ketamine leads to inhibition of active GSK3β as part of its mechanism of action. The issue with wide use of ketamine is that ketamine itself also causes psychotomimetic symptoms when given even in low doses to humans. However, it is possible that a specific and selective GSK3β inhibitor could work like ketamine without the adverse side effect.

We have discovered a novel structure highly selective for GSK3β and our chemistry efforts in 2012 will focus on making a compound with sufficient pharmacokinetic properties and selectivity for this purpose. See the Wnt Project Report (section 3) for more details. We would then seek a way (via other external funding) to take this compound into clinical trials modeled on the ketamine trials.

**Clozaril Mimetic**

Clozaril remains a unique antipsychotic medicine but it is still confounded by serious side effects. Dr. Marc Caron of Duke University and his colleagues have published that clozaril is unique in blocking only the β-arrestin-mediated (and not cAMP) intracellular signaling pathway from the dopamine 2 receptor (‘D2R’) (Masri et al (2008) PNAS 105:13656). In close collaboration with Michelle Palmer’s group in the Chemical Biology Platform at the Broad, we developed over the past 18 months screens to look for new structures that would have this effect on D2R signaling. As described in the separate report on our ‘PsychHTS Initiative’, our efforts have been successful and we have compounds that seem to block this arm of the D2R pathway and not the cAMP arm of this pathway. In Caron’s work, mice lacking the gene for β-arrestin2 are resistant to amphetamine-induced hyperactivity. Thus, the hypothesis is that selectively blocking this signaling arm of the D2 receptor pathway would have antipsychotic clinical benefit.

Published biochemical analyses done in vivo in mouse striatum show that amphetamine administration leads to a decrease in the phospho-Ser308 form of Akt1, a key enzyme controlling the signaling in that arm of the D2R/β-arrestin mediated pathway. We have discovered that NO ONE has shown this important result in a cultured cell. We are working diligently to develop such a cell-based assay to monitor the functional effects in cell culture of
such a selective antagonist of D2R signaling. This key assay will greatly accelerate the progress that can be made in this project. Once we have done this, we will initiate a serious chemistry effort to make a D2R/β-arrestin pathway selective antagonist. The possible outcomes of this effort could be a molecule that mimics Clozaril fully in efficacy but free of its side effects (which are certainly off-target effects), or at least a much better tolerated antipsychotic. We are hoping to have such a functional cell culture assay by the end of 2Q 2012.

**Synaptic assays**

We hope to have such an assay as noted above in place in 2012 and begin chemical screening. The best method for doing this in a high throughput mode is being determined by Dr. Feng.

**5. TRANSLATIONAL EFFORTS**

As the Stanley Center has evolved, we are increasing our efforts to conceive of, promote and support projects that can translate our discoveries to the clinic, for both diagnostic and therapeutic purposes. In 2010 we formalized this goal by adding a Clinical Project Team, headed by Dr. Roy Perlis, to our handful of focused project teams. Below I summarize two key types of these translational initiatives.

**LOGIC: A Study to Translate Psychiatric Genetic Findings to a Pediatric Clinic**

A program was initiated in late 2009 with the MGH Department of Psychiatry to obtain samples from children with various behavioral and cognitive problems seen in the clinic. Some initial seed funding was provided by the Stanley Center. The Longitudinal Study of Genetic Influences on Cognition (LOGIC) has now established a firm foundation in its second year that will promote its continued growth and ability to secure outside funding. Dr. Alysa Doyle, a licensed child and adult clinical psychologist with expertise in pediatric neuropsychology and psychiatric genetics, leads the project. It is described in more detail in the Patient Collection Report; however, I am highlighting it here because I believe it is a unique effort with fantastic long-term potential for psychiatric research and clinical practice, perhaps akin to the Framingham Heart Study’s contribution to understanding heart disease.

LOGIC takes a novel, developmental approach to translational genomics by aiming to collect DNA and extensive phenotypic information from 3,000 youth evaluated at a pediatric neuropsychology clinic within the MGH Psychiatry Department and then to follow the sample over time. The study intentionally does not bias for or against any types of behaviors or diagnoses. Instead, by virtue of the concerns that are driving these patients’ referrals (i.e. problems with cognitive, language, motor, social and adaptive functions), these youth are at neurobehavioral high risk for major mental illness in adulthood, including schizophrenia. This cohort thus represents a broad spectrum of potential psychiatric disorders. The overarching goals of the project are to translate recent findings from the field of psychiatric genetics to the clinic and map trajectories to severe adult psychiatric illness. At baseline, the study will examine high-priority genetic variants from the field in a child clinical cohort (including rare, risk-conferring CNVs as well as genome-wide significant common variants and polygenic signatures from PGC and Stanley Center schizophrenia, bipolar and cross-disorder analyses). Over the next decade, investigators will amass a large, longitudinal evidence-base to disentangle complex etiologies, improve personalized risk assessment and identify opportunities for intervention. Such programmatic work is the next logical step for the field given advances in
gene discovery for schizophrenia and bipolar disorder and the shared risk of these conditions with autism spectrum disorders and ADHD.

Preliminary phenotype data (from over 390 probands and their families) and genotype data (from 74 cases run on an Illumina 2.5 v1 array (with over 2 million SNPs) already confirm that the sample will be an important resource for the Stanley Center and the entire field. Details are further described (see the LOGIC section of the Patient Collection Report), but two specific findings are of particular note. First, preliminary data also confirmed the clinical relevance of overall levels of structural variation in the LOGIC sample. For example, although overall CNV size was not larger in severe cases within the sample, large CNVs (> 500kb) were of significantly greater size in individuals who had been hospitalized and in cases who exhibited psychosis versus cases who did not (Figure 4). Second, these early data also demonstrated the relevance of the schizophrenia polygene score to the LOGIC sample. For some bins, there was nominally significant evidence of enrichment in the LOGIC sample compared to controls (detailed in the Patient Collection Report). It is actually striking, with a sample number less than 100, to find even some significant enrichment in the polygene score, and this low power is a likely reason for both the nominal significance and the absence of enrichment at some thresholds. These preliminary results support the hypothesis that ascertaining children from this clinic is enriching for individuals who share genetic risk for serious neuropsychiatric disease in adulthood including schizophrenia. As both the number of genotyped samples increases and the cohort is followed longitudinally, the relevance of the cohort and the predictive value of these genetic analyses will be tested.

![Figure 4](image)

**Figure 4.** A higher burden of large CNVs in the LOGIC patients is associated with either psychiatric hospitalization (A) or psychosis (B). CNV size is >500kb.

**Clinical (Therapeutic) Work**

The knowledge gained from identifying a disease risk gene can often be translated into novel therapeutics. Admittedly, sometimes the discovery of disease-causing gene variants does not rapidly lead to new therapeutics. Examples such as Huntington’s disease and Parkinson’s disease are such disappointing examples.

However, it is clear that in some fields there are wonderful success stories. For single gene diseases, the discovery of the mutation in the Fibrillin gene, the cause of Marfan’s syndrome (Dietz et al (1992) Hum Mutat 1:366), led to the elucidation of the biological pathway
affected. This insight led to the use of Cozaar, a drug which dramatically reverses and stabilizes the aortic aneurysm process that otherwise can be fatal in patients with Marfan’s syndrome. For Cystic Fibrosis, the FDA is reviewing a drug that actually significantly improves the function of a common mutant form of the cystic fibrosis gene product. Lastly, mGluR5 antagonists have shown clinical benefit in a rare form of autism spectrum disease, Fragile X syndrome. In this case, as in Marfan’s, the discovery of the gene that causes Fragile X syndrome led first to an understanding of the pathophysiology of the disease and then to a treatment mechanism which reverses that pathophysiology—even while the mutant gene is functionally defective in the patient.

Examples also exist in diseases where common variants have been discovered that confer a small degree of risk. Hypercholesterolemia and type II diabetes are prime examples where common variants identified through GWAS may point directly at therapeutic targets. In the case of statins, which inhibit HMG CoA reductase, the Mendelian mutation that leads to hypercholesterolemia is in the LDL receptor, yet the treatment point in the pathway is an enzyme that has a common low-penetrant risk variant (Kathiresan et al (2008) Nat Genet 40: 189). In Type 2 diabetes a low-risk variant, the PPAR gamma receptor, is the target of two glitazones which are useful treatments for type 2 diabetes.

At this time in psychiatric genetics, clear Mendelian mutations (100% penetrant) have not been discovered. Indeed, from epidemiology studies, such mutations would not be expected, or would be quite rare. However two sources of information do exist to stimulate new approaches to treatment. First, exome sequencing, now a major focus in the field, is beginning to find promising candidate genes of higher penetrance. Second, GWAS studies reveal association signals that point toward a particular gene. Often these ‘hits’ are located in intergenic regions spanned by multiple plausible disease genes. Yet when the signal lies in a single gene—even if the causal variant is not identified—it may still identify a possible treatment target. A key initial question is whether the disease-associated variant causes a gain or loss of function. While this can be inferred in some cases using in silico tools, it is likely to require investigation in cellular models. These same models can then be applied to screen small molecules or other interventions that reverse this effect, or its downstream consequences.

The path to development can take one of two routes. The first is development of a screen, discovery of lead compounds, optimization of these compounds, toxicology testing and eventually clinical trials— a long and arduous process. Despite the daunting odds against success, this process can ultimately yield a novel compound which can result in an FDA-approved therapeutic some years later. We clearly are pursuing this approach in our Center: The HDAC, GSK3β, and Clozaril mimetic projects are the current examples.

The second path is to examine FDA-approved medications to identify a compound with known safety in humans, hoping that the effect of the compound on the target gene or pathway will be beneficial in a disease for which the drug was not initially developed. This approach should still firmly be based upon scientific hypotheses that emerge from the genetics. In this case, the process of clinical translation could be dramatically accelerated. Safety and efficacy are known from the prior development data, but must still be established in human trials, in the new disease. The better grounded the hypothesis is, deriving from the underlying genetics and biology, the greater the probability of success. The Clinical Project Team Report by Dr. Roy Perlis elaborates on four such cases that the Stanley Center. I summarize the key points below.
1. **Isradipine for bipolar depression**

   The SC genetics program used GWAS to first identify the gene CACNA1C, coding for the alpha subunit of the L type voltage-gated calcium channel, as a risk gene in bipolar illness and, subsequently, in schizophrenia. The exact functional or nucleotide variation within the channel gene has not yet been identified. In principle, changes in CACNA1C could contribute to either increase or decrease in calcium currents, so the desired therapeutic mechanism was not immediately apparent. However, as there are multiple families of calcium channel antagonists with FDA approval for hypertension, Dr. Roy Perlis conducted a thorough review of all these compounds’ properties and performance in all clinical trials, and drew the following three key conclusions: 1) There was no evidence that calcium channel blockers (CCB’s) would exacerbate the course of bipolar disorder, so they appeared to be safe from a psychiatric perspective. 2) There was little known about the potential efficacy of CCB’s in bipolar depression, an area of substantial clinical need, suggesting that this might be an important aspect of the illness to investigate. 3) Many of the bipolar trials that had failed to demonstrate efficacy used CCB’s that are not specific for L-type calcium channels, suggesting that more specific treatments might yield a better balance of efficacy and tolerability. Dr. Perlis identified isradipine as having an excellent safety profile (such that it was commonly used for pediatric hypertension), good CNS penetration, and slight selectivity for L-type calcium channels.

   Thus, the clinical team conducted a proof-of-concept study (10 patients), for which an interim analysis was presented in the 2010 report. This study, detailed in the Clinical Report, has now been completed with encouraging positive, though, preliminary, results. Based on the results of this pilot investigation, Dr. Perlis obtained funding from the Stanley Medical Research Institute in November 2011 for a randomized, controlled investigation of adjunctive isradipine in bipolar depression. This single-site study will investigate isradipine in 30 bipolar I, depressed subjects without a history of hypertension or cardiovascular disease. The first patient is anticipated to enter the trial in January 2012, with last patient exiting in January 2014. All subjects will provide DNA, fibroblasts (from 1.5 mm skin biopsy), and lymphocyte samples (from whole blood) for further investigation of cellular phenotypes.

2. **Statins as Augmentation to Lithium Treatment for Bipolar Depression**

   A second proof-of-concept study was motivated by data (both by SC scientists and others) indicating potential synergy between some HMG-CoA reductase inhibitors and lithium, in terms of effects on Wnt signaling in cellular models. A rat model likewise suggested an antidepressant-like effect in the forced swim test. Again, this hypothesis was tested and strengthened by combining approaches and ideas grounded on the possible mechanism to test this hypothesis. Dr. Haggarty conducted *in vitro* studies that showed HMG-CoA reductase inhibition enhanced Wnt signaling and, perhaps more important, that this affect was additive with lithium. Dr. Perlis used ‘i2b2’—Informatics for Integrating Biology and the Bedside, an NIH-funded National Center for Biomedical Computing based at Partners HealthCare System—to query the large database of medical records of patients prescribed both antidepressants and statins, and found potential benefit from statin augmentation.

   Drs. Perlis and Haggarty recently obtained NIMH R21 funding (November 2011) for a single-site R21 incorporating a proof-of-concept randomized clinical trial examining simvastatin 20mg or placebo as add-on to lithium. This study will enroll 24 individuals with bipolar I or II depression, using the sequential parallel comparison design (Fava) in which subjects who fail to respond to 4 weeks of treatment are re-randomized. All subjects will provide samples to be used *in vitro* to examine Wnt signaling and to identify correlates of *in vivo* treatment response. The first patient is anticipated to enter in February 2012, with last patient exiting in January 2014. This study and its rationale are described in more detail in the Clinical Report.
3. **GSK3β inhibitors for depression**
   
   I already outlined above (under Chemical Screens) the idea behind this potential third proof-of-concept clinical study. The rationale is that ketamine’s mechanism of action in affecting relief of treatment-resistant depression is in part its inhibition of GSK3β. If this mechanism is indeed true, then a specific and selective GSK3β inhibitor might work like ketamine without the adverse side effect. Our GSK3β inhibitor project has identified such a potentially novel and highly selective compound. Thus a key milestone for our chemistry efforts in 2012 will focus on making a compound with sufficient pharmacokinetic properties and selectivity to obtain an IND (investigational new drug) approval from the FDA, allowing a proof-of-concept investigation of a novel GSK3 inhibitor instead of ketamine. Planning for this pilot will be informed by the two randomized controlled trials above (isradipine and statin augmentation) as well as the published ketamine trials.

4. **PDE4 inhibitors alone or as adjunct therapy with common antidepressants for depression**
   
   This last potential clinical study is based on recent striking biochemical results from Dr. Li-Huei Tsai’s lab, detailed in the Wnt Report. While investigating DISC1 interacting proteins, they found that DISC1 binds DNA via its interaction with the transcription factor ATF4. They focused their studies on the DNA binding region within the phosphodiesterase 4D (PDE4D9) locus. (DISC1 has also been shown to associate with protein members of the PDE4 family, and this association is believed to play a role in DISC1 regulation of affect and cognition.) The phosphodiesterases are the sole means of converting the cyclic forms of AMP and GMP to their monomeric forms. The PDE4 family, in particular, is responsive to rolipram, which demonstrates antidepressant properties in rodents, but is not approved for human use in the United States because of its significant adverse effects, particularly nausea. The Tsai lab first showed that showed that DISC1 and ATF4 form a repressor complex that specifically represses expression of the PDE4D9 isoform. Dysfunction of DISC1 (as in the Scottish family with the DISC1 translocation and with the high incidence of depression, bipolar disorder and schizophrenia) leads to a dramatic increase in levels of PDE4D9. Subsequent work revealed that D1 (but not D2) dopamine receptor stimulation could recapitulate this effect of DISC1 loss of function. Overall their findings have led to a proposed model in which increases in PDE4D9 expression following the activation of catecholaminergic receptors and downstream PKA signaling cascades serve as a transcription-mediated negative feedback loop. Hence, reducing PDE4D9 levels and/or activity might be antidepressant. This possible mechanism of PDE4D9 inhibitors, combined with previous published reports of rolipram as a monotherapy for depression and that rolipram’s side effects are dose-dependent in animals, leads to the hypothesis that low-dose rolipram might have an additive antidepressant effect when combined with other antidepressants.

   The Tsai lab plans to investigate the effects of combinatorial therapy using rolipram and SSRIs, (compared to SSRI treatment alone) on mouse models of depressive behavior. If further evidence of efficacy for the combination is obtained, we will plan a proof-of-concept investigation of roflumilast adjunctive to standard antidepressants in mood disorder patients, for which we would seek external funding in Q2-3 2012.

   In summary, these four small studies reflect various stages of following the ‘second path’ to development of novel therapeutics for psychiatric disease. We believe such translational efforts may lead to valuable insights for treatment, and complement other, longer efforts of drug discovery following the ‘first path’ of chemical screening of novel targets or pathways.
6. OPERATIONS OVERVIEW

Productive focused research is made possible by optimal organizational structure, leaders, and management practices. I already noted our important organizational and management changes. This section highlights, or reiterates from past reports, some other key points.

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 evolved over these five years. After last year’s annual Meeting in January 2011, we made several changes. In particular, we needed to address reasonable terms for such appointments, as we had imposed on some members for over four continuous years. We decided that rotating about half of our SAB members with 4-year terms would allow us both continuity and rejuvenation. Thus, this past year we have added several new members and have said thanks and farewell to a few others. In addition, we made one change in our Operating Committee: Since Dr. Steve Hyman came to the Broad in July 2011 for sabbatical, it seemed inappropriate to have him continue as one of the three required Harvard faculty on our Operating Committee. We are pleased and honored that Dr. Christopher T. Walsh of the Biological Chemistry and Molecular Pharmacology Department at Harvard Medical School kindly accepted our invitation. The current list of all members of our Governing Committees is in Appendix A. Given the rapidly evolving types of genes and pathways being identified by the genetics (such as ubiquitination and synapse transmission), we also continue to seek specific experts for advice on an ad hoc basis.

As evidenced by the structure of this Annual Report, with emphasis on Project Team Reports, the core functional units of the Stanley Center remain our cross-discipline Project Teams. In past Reports I have noted that the Project Team meetings provide an opportune forum in which the multidisciplinary interactions essential to our mission can occur. The goal is to generate ideas that can extend the boundaries of what individual investigators might think about or can affect experimentally. These 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. Logistically, we rotate weekly meetings, so that each Project Team meets about once every two to three months. 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 identified by the genetics or neurobiology programs and to accelerate our overall research progress. 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, a full-day meeting here at the Broad in June 2011, and many CSHL members attended our recent 2-day Symposium in September. We will aim for our next meeting at CSHL in 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 (‘10 – ‘11) and the current ’11- ’12 academic year (see Appendix B) reflect the breadth and quality of topics and speakers.

In closing, we want to mention the amazingly engaging and enlightening conference we had this past September. The ‘Symposium on the Emerging Genetics & Neurobiology of Severe Mental Illness’, held on September 22-23, 2011, brought together almost 350 leading scientists working on emerging genetics of schizophrenia, bipolar disorder, autism and other mental illnesses. The sessions covered the full arc of research in the field, from genetics to functional neurobiology to clinical applications. A testament to its usefulness and success was the overwhelming response (>30% of attendees) to our online survey, with most saying they found it highly informative and beneficial, and >95% saying they would attend another similar conference in two years. A summary is provided in Appendix E.