STANLEY CENTER

FOR PSYCHIATRIC RESEARCH

2nd Annual Report

January 2009

EXECUTIVE SUMMARY

The second full year of the Stanley Center for Psychiatric Research has been an exciting and highly successful year. Novel and clinically relevant discoveries in the Genetics program point a clear path forward towards an eventual full understanding of the genetic causes of schizophrenia and bipolar illness. *In addition, the findings lay the ground work for the first quantitative test that will be clinically useful in diagnoses of any severe mental illness.*

The genetics work is highlighted in the summary below. Consistent with the mission of the Stanley Center for Psychiatric Research, namely to develop novel diagnostics or therapeutics for schizophrenia or bipolar disorder based on a genetic understanding of the diseases, the genetics findings are clearly most fundamental at this early stage of the Stanley Center. We anticipate the results of the Genetics Analyses will drive the future focus of all our research programs. The neurobiology, behavioral biology and medicinal chemistry programs have made good progress on two targets for potential new treatments. This work is also summarized below. Importantly, we are in discussions with both established pharma companies and venture groups on how to accelerate progress in each of these two drug discovery projects so that we increase the chances that tangible benefit will come from the work.

After this Executive Summary, you will find separate Progress Reports written by the Director of each of the core Stanley Center disciplines (Genetics, Chemical Neurobiology, Neurobiology, Behavioral Neurogenetics, and Medicinal Chemistry). These Program Progress Reports are more technically detailed than this Summary on the scientific research within each group. The appendices capture other types of information essential to Stanley Center operations (personnel, collaborators, our seminar series, intellectual property, publications, expenses and budgets), and they are summarized at the end of this Executive Summary. The scientific research is so exciting we are compelled to begin with it.

Finally, based upon our experience in the past two years, and the equally important progress in the Broad Institute's Chemical Biology and Novel Therapeutics programs, we propose an expansion for the chemical program we currently have, in order to organize a more global effort to find new treatments. This would require additional funding just for this new effort as discussed with Julie Friese, Fuller Torrey, and Mike Knable. A separate document detailing this proposed initiative is included with this Annual Report. We have proven we can organize a global genetics effort in the first two years and this collaborative effort has totally changed the direction of work in psychiatric genetics. We want now to do the same for drug discovery in this field with the aim of showing pharma companies what to work on for new therapies.

I. GENETICS FINDINGS

Bipolar Illness

In our first year 2007-2008, we identified two risk genes for Bipolar Illness: ANK3, encoding the Ankyrin-G protein, and CACNA1C, encoding a protein component of a calcium channel which allows calcium ions to flow in and out. Ankyrin-G plays a role in the development of inhibitory interneurons--how they connect to and regulate the firing of neurons-- and in how neurons develop their own electrical current systems (at Nodes of Ranvier). We have begun work to identify the exact molecular defect(s) associated with bipolar illness based on these genetic results. Calcium channels are crucial in how neurons fire and we have also begun work to identify the variant of this channel associated with bipolar illness. Based upon the calcium channel finding, a small clinical

study is underway by Dr. Roy Perlis of MGH to see if a brain penetrant calcium channel blocker (Isradipine) can be beneficial in bipolar illness. It is also worth SMRI considering a trial of Lyrica for this illness since this drug is both brain penetrant and a different kind of calcium channel blocker.

As we discussed in last year's Annual Report, there is a critical need for larger patient samples in studying the genetics of this illness. With the advancement earlier this year (2008) in Stanley Center funds and funds obtained from NIMH (partly due to the Stanley Center funding they knew about) we have a consortium now collecting more samples. For the Stanley Center-funded effort, 800 total cases to date have been collected from initial efforts; over 2500 additional samples are expected by end of 2009 (for total ~3500); over 5000 more by end of 2010; the goal of 10,000 cases to be reached in 2011. The NIMH-funded collection will yield an additional 10,000 cases and 10,000 controls by 2013. More details of this effort are described in Appendix A.

Schizophrenia

Gene Deletions

An important finding from the International Schizophrenia Consortium (led by Stanley Center scientists and partially funded by the Stanley Center),is that there is an excess of gene deletions in patients with schizophrenia vs. controls. This is a hallmark finding in the field. The increased burden in cases is small but it is absolutely certain. The finding has been reproduced in three other laboratories using different approaches.

Among the deletions are large ones that are found on chromosomes 1, 15, 16, and 22, in each case removing several genes. These large deletions account for approximately 1% of the genetic basis of schizophrenia. In addition, single gene deletions are also in excess. Work is in progress to identify which genes are specifically deleted in patients vs. those deletions found as normal background in controls. An analysis suggests that several genes important for brain development are among the specific ones deleted in schizophrenia patients. The best evidence so far suggests that some specific deletions are occurring de novo during the development of a new embryo, although some deletions may already exist in a parent and are passed on to the new child. Work in the next 1-2 years will attempt to elucidate which genes play key roles in pathogenesis of the illness, thereby leading to both development of new diagnostic tests and ideas for treatment based upon the pathways that are functioning abnormally. As noted below, we believe we have identified, even now, *one metabolic pathway that is awry and contributes to the etiology of schizophrenia and bipolar illness.*

Common Variants

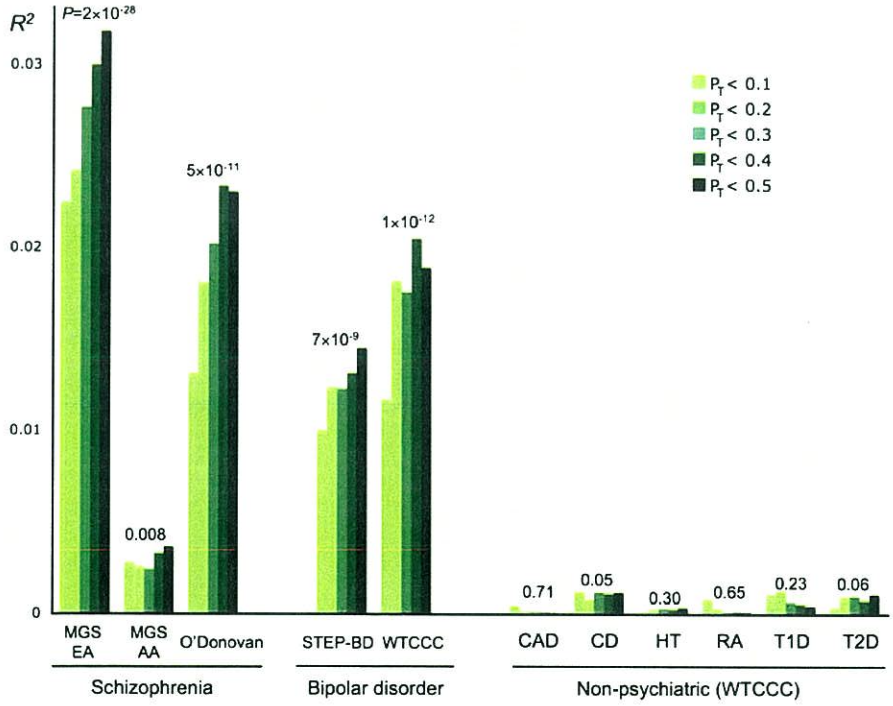
In general, the human population has 7-10 common variants of each of the ~ 30,000 human genes. Work in human genetics in the past 3 years has identified, for several human diseases where genetic risk is involved, commonly occurring variations in the single DNA nucleotide bases and which of these variants (and in which gene(s)) confers risk to the cause of the disease. Examples of diseases in which several common variants have been discovered include age-related macular degeneration, Crohn's disease and ulcerative colitis, type 2 diabetes, prostate cancer, breast cancer and many others. These studies, by investigators not in psychiatric genetics, have found many new genes involved in these diseases, some of which are already being worked on in both academic laboratories and companies aiming to discover new treatments. In each case, many genes are involved in determining disease risk. An even more extreme example has come from studies carried out to find genes that control height in humans.

In this work on height in humans, the key finding is that *hundreds of genes with small effects of each gene* are involved in determining a human's height.

Dr. Shaun Purcell and Dr. Pamela Sklar in our Stanley Center have approached. the question of whether a similar mechanism--many genes, each with a small effect--contributes to the etiology of schizophrenia, to complement both their efforts to identify specific genes and their findings about gene deletions. They developed a *polygene scoring system* to carry out this work and the results are spectacular.

Their analysis finds that a polygene score representing thousands of bases in DNA and hundreds or more genes provides a *genetic signature* that distinguishes a population of patients with schizophrenia from a population of controls. The polygene score has correctly identified three other collections of samples from patients with schizophrenia, and we will describe a fourth when we meet with the SMRI members on January 15. Amazingly, as shown in Figure 1, the score also identifies two separate populations of samples from patients with bipolar illness while it does *not* identify six other nonpsychiatric illness samples (and at least one other to be discussed in person).

The results are easy to see in the bar graph in Figure 1. The score currently represents a mixture of genes that are giving the signal and genes that are not involved and hence are diluting the signal. The data have been garnered from approximately 3300 cases and 3600 controls. As Shaun and Pamela analyze data from approximately 10,000 cases, the score will be refined and will be much better in its specificity and sensitivity. The refinement should be possible in the next 6-12 months.**Figure 1.**



Application of 'polygene score' derived from schizophrenia GWAS to genotype data from other large patient population samples for other diseases. Abbreviations: R^2= polygene score; P_T= Probability threshold value of genes used for scoring; MGS =Molecular Genetics Consortium Samples-- 'EA'=European-American or 'AA'=African-American; O'Donovan= UK sample reported in Nature Genetics '08); STEP-BD=Systematic Treatment Program for Bipolar Disorder; WTCCC=Wellcome Trust Case Control Consortium study reported in Nature '07 on 2000 patients in each of 7 diseases, plus 3000 controls; CAD = Coronary Artery Disease; CD= Crohn's Disease; HT=Hypertension; RA=Rheumatoid Arthritis; T1D=Type 1 Diabetes; T2D= ype 2 Diabetes.

What Do These Findings Mean For The Etiology Of The Illnesses?

First, the data unambiguously indicate common genetic variation plays a role in the causes of both illnesses. That bipolar illness and schizophrenia share some common genetics has been hypothesized but this is the first clear proof. Second, a plausible model for the genetic cause of schizophrenia is analogous to our current understanding of many cancers: that a person with some set of these common variants has an additional insult, like a gene deletion arising during the development of the embryo or passed on from an asymptomatic parent, and this combination (common variants plus additional insult) leads to the illness. An example of another second insult might be a viral infection during pregnancy. Third and very important, in preliminary data, patients with bipolar illness do NOT carry an excess of gene deletions like those detected in schizophrenia. Thus the two diseases do not have an identical genetic underpinning. With analysis of additional cases, it should be possible to refine the score for better definition and ideally, the separation of bipolar illness from schizophrenia.

As this genetic polygene score is refined, many important clinical questions will be asked:

Does the score correlate to certain symptoms, to age of onset, to drug response, to cognitive deficits, to psychotic symptoms? Can a group at high risk based upon the test be identified in which a prevention study can be carried out? What genes are being identified and how can that information be translated into new screens for new therapies? In time, a DSM (Diagnostic and Statistical Manual of Mental Disorders) will use such tests to construct new diagnostic categories.

Association Data In Schizophrenia

One salient finding about a gene group that has been found in the schizophrenia studies so far has emerged. Variations in the HLA gene locus are protective in our studies against schizophrenia. This region of the genome, also referred to as the Major Histocompatibility Complex or MHC, is indeed very complex and contains a large number of genes related to immune function. We will need to consult experts in this field to decide how to try to unravel the meaning of this intriguing discovery. Because we know the result is reproducible, it is definitely worth pursuing.

In the coming year we will refine the polygene score as noted above, and begin sequencing efforts to identify exact gene variations within the genes involved in the etiology of bipolar illness and schizophrenia. The DNA sequencing strategy will be developed with experts at the Broad Institute, as technologies available for cheaper large scale gene sequencing are evolving rapidly. Being located at the Broad Institute provides us the unique opportunity to determine, choose, and quickly apply the best and most appropriate technologies to our specific questions. New methods will also be available to detect smaller gene deletions and we will utilize these as needed. Further details are described in the Genetics Program Progress Report.

II. NEUROBIOLOGY, BEHAVIORAL BIOLOGY, AND CHEMISTRY: TWO DRUG DISCOVERY PROJECTS

The individual progress reports that immediately follow this Executive Summary describe in some detail the findings of each Stanley Center discipline separately. This summary integrates the findings to highlight specific progress towards the discovery of new targets and new approaches to therapy, and to note the progress in chemistry towards that goal.

DISC1 Gene Pathway And Gsk3 Beta As Drug Target

Work from the Tsai and Sive laboratories has discovered a major role for the DISC1 gene in brain development. The DISC1 gene was discovered 8 years ago in an extended Scottish family beset by severe mental illness. By tracking the disruption of this gene in the family and members of the family affected with severe depression or psychotic illness, scientists showed that the disruption of this gene in this family plays an important role in the pathogenesis of such illnesses. The scientific community has published many papers on the biology and biochemistry of the Disc1 protein in the past eight years. But in the past 18 months Tsai and Sive in collaboration with the Stanley Center and each other, have added an important new fact to the puzzle. It is important to appreciate that the biological role of DISC1 has emerged from work in mice by Dr. Tsai and in zebrafish by Dr. Sive. Thus, two independent biological models have demonstrated that *the DISC1 gene is involved in regulating a major pathway involved in brain development.* The pathway is known as the *Wnt signaling pathway*, named for the protein hormone that regulates it by binding to its receptor on the surface of brain cells during development. Importantly the major place in the pathway in which Disc1 acts has also been found: The Disc1 protein regulates the enzyme activity of Glycogen Synthase Kinase 3-beta (GSK3-β), a key enzyme involved in regulating the Wnt pathway. This is the same enzyme regulated by lithium. The detailed biochemistry by which lithium inhibits this enzyme differs from the detailed biochemistry by which Disc1 regulates GSK3-β.

The coincidence of these two findings is very exciting because it has therapeutic implications. Inhibiting GSK3-β has been shown in animals to ameliorate amphetamine-induced hyperactivity, one of the behavioral models by which antipsychotic therapies have been found in the past two decades. The Stanley Center Behavioral group has become functional in the past year after the lab animal space renovation was finished in January 2008. They have reproduced the amphetamine model and the effect of GSK3-β inhibitors (see Figure 2, included here to highlight the quality of their work and the robustness of the model.). They also have developed a novel quantitative assay for this model in collaboration with a world class imaging group at the MGH. We have begun a project to discover a drug that inhibits this enzyme in a novel way so that the drug can selectively act at this enzyme. This project is based upon discoveries about how Disc1 inhibits the enzyme. An interdisciplinary project involving structural work on the enzyme, chemical screening, biochemistry, chemistry and animal work is underway. A large pharma company seems very interested in collaborating with us in this project. We hope to effect such an agreement soon and thus be able to expand our efforts and improve the chance that practical results can emerge. We think this working model will be an efficient way to stimulate new drug therapies in the field: i.e., we make the discoveries and establish first proof of principle in a new direction and then find a pharma partner to take on the program. This is a good model for accelerating the rate at which new therapies can be discovered and developed.

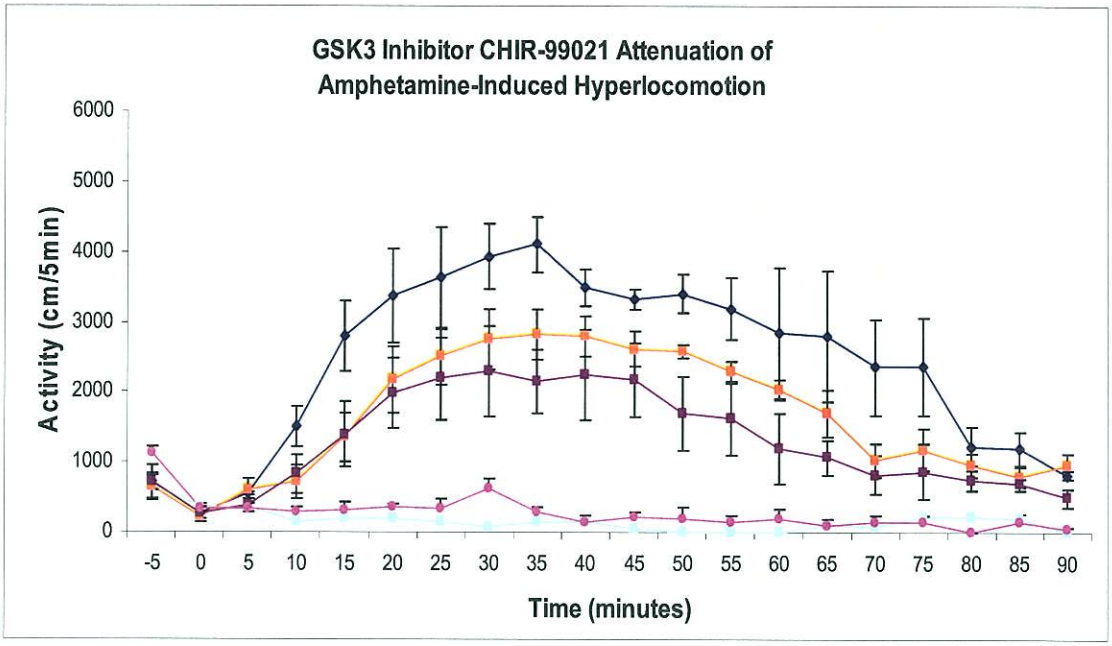


Figure 2. GSK3 Inhibitor CHIR-99021 attenuates amphetamine-induced hyperlocomotion (a model for mania). CHIR-99021 was administered by direct brain intracerebroventricular infusion, followed by immediate placement of the animals in the testing arena. Hyperlocomotion induced by amphetamine (3.5 mg/kg at Time 0; **dark blue**) was significantly reduced by CHIR-99021 at 0.1mg/ml (**orange**) and 1.4mg/ml (**purple**), corresponding to a physiological concentration of 7uM throughout brain based on total CSF volume or total brain volume, respectively. No effect on baseline activity was observed during the 10min period prior to amphetamine challenge, or for a higher 14 mg/ml dose (light blue) compared to 100% DMSO vehicle (pink) over the entire activity period, suggesting the AIH attenuation by the 0.1mg/ml and 1.4mg/ml doses are not due to non-specific motor effects. N = 3 to 4 mice tested per group.

Histone Deacetylase (HDAC) Inhibitors

The second major chemical biology effort is focused upon histone deacetylases ('HDACs') as targets for new treatments. This family of enzymes has 11 distinct functional members, each involved in regulating chromatin structure and gene expression. These 11 enzymes are divided into two separate structural and functional subgroups (Class I and Class II). Thus a large and sophisticated effort is required to find selective and safe inhibitors of a designated member of a single class. Building upon prior work at the Broad Institute we have established a drug discovery effort aimed at 1-2 members of this family of enzymes. Work in the Tsai laboratory has shown that HDAC2 (a member of Class I) is a good target for improving cognitive function (a major problem in schizophrenia). In addition, work in the Haggarty group and the Petryshen Behavioral group has shown that inhibiting a Class I HDAC may be a way to ameliorate amphetamine-induced hyperactivity. It is not clear at this point which Class I HDAC is the best target for this latter utility. The chemistry group has made about 200 compounds in the past year and molecular modeled HDAC2. Reasonable progress has been made towards identifying more selective, safer and potent inhibitors, and animal tests are underway on some of these compounds. Insuring that the compounds enter the brain and have sufficient potency and half life have been part of this effort. We are in active discussions with pharma companies and venture groups to obtain the resources to expand this program and improve the chances of practical success.

III. SUMMARY OF TRANSLATIONAL EFFORTS AND PLANS

The following are relevant translational work that has come from the work of the Stanley Center in the first two years.

1. Identification of drug targets and progress towards therapy
    a. Ion channel role in bipolar illness. Work to elucidate molecular basis of both Ankyrin-G and calcium channels is underway. Pilot clinical study in bipolar illness at MGH with brain-penetrant calcium channel blocker is in progress.
    b. Major program towards a novel inhibitor of GSK3-β. Discussion with pharma is ongoing to accelerate project.
    c. Major program on HDAC inhibitors for memory (cognition, relevant for schizophrenia) and mania bipolar illness. Current primary focus of Stanley Center's Medicinal Chemistry group, with compounds made, and modeling and both in vitro and in vivo testing of these compounds in progress. Discussions with outside companies to allow acceleration of program.
2. **Development of a 'polygene score' for detecting genes involved in schizophrenia and bipolar illness. Enormous promise towards clinical tests, and new targets. First evidence for a quantitative test for these two illnesses. First quantitative test ever in the field of psychiatry.**
3. Discovery of excess burden of gene deletions in patients with schizophrenia. Progress towards which genes are involved in disease pathogenesis.
4. Identifying a signaling pathway that appears to be involved in causing both illnesses and thus focusing our research on this pathway for possible new treatments. Figure 3 below depicts some of the major effectors in this Wnt signaling pathway, highlighting in red boxes the steps in the pathway that implicate the Wnt signaling pathway in the pathogenesis of schizophrenia and bipolar illness.

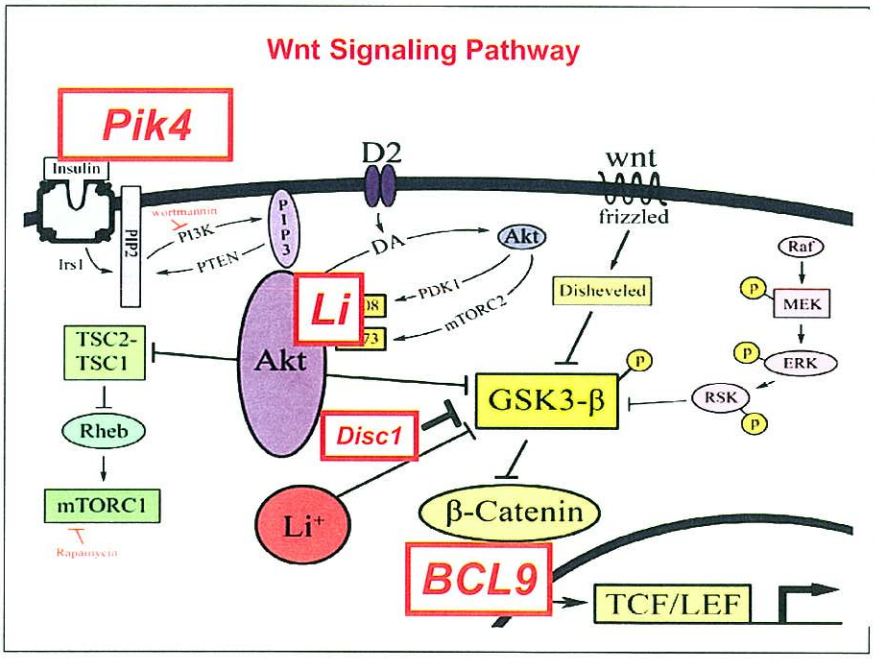


Figure 3. Diagram of the Wnt Signaling Pathway. Red boxes highlight proteins (Pik4, Disc1, Bcl9) and the therapeutic lithium (Li) that act at the steps indicated by their relative location and that implicate this pathway in both bipolar disorder and schizophrenia. More details of the evidence and mechanisms are described in the Genetics, Neurobiology and Chemical Biology Program Progress Reports.

IV. STANLEY CENTER OPERATIONS OVERVIEW

Productive focused research is made possible by good organizational structure and procedures, beginning with personnel. The summary organizational chart for the Stanley Center shown in Appendix B1 highlights the status within each disciplinary Program, Broad Platform, and collaborators. Some key positions included in this summary are planned to be filled in 2009. Of these, it is worth mentioning the plan to support chemical biologists who will be dedicated to providing the needed routine screening support for the HDAC and (eventually) GSK3- projects. In response to feedback from our Operating and Scientific Advisory Committees after our August '08 meeting (concern that our in vivo/ behavior operation was weak), we have now added Dr. Michela Gallagher (Professor, and Head of the Neurogenetics and Behavior Center, at Johns Hopkins) to our SAB, and we plan to hire two additional BS/MS behaviorists (one under Dr. Tsai and one under Dr. Petryshen) as well as a full-time histologist. We also realize that a full-time permanent staff position (computational genetics) in the Genetics group would provide critical continuity to the Program instead of relying primarily on post-doctoral associates. Finally, to help attract and nurture cross-disciplinary researchers particularly interested in translational research for psychiatric disease, we are planning to establish a Stanley Center Fellowship. This fellowship would require that the candidate work on a project encompassing at least two of the five core disciplines within the Stanley Center; the appointment would last 3 years, and we anticipate appointing one candidate a year. The essential cross-discipline nature of our core functional units (our Project Teams) is seen in Appendix B2.

To stay informed and responsive to the rapid pace of research, especially with genetic studies that identify genes out of our area of expertise (such as ANK3 or MHC), we use our Stanley Center Seminar Series to explore new ideas and fields, potential collaborations, and alternative perspectives, all immediately relevant to our Stanley Center mission. The seminar schedule from last year (Appendix C1) and the current '08- '09 academic year (Appendix C2) reflect the breadth and quality of topics.

Collaborations are a key means to pursue discoveries identified by the genetics or neurobiology programs and accelerate our overall research progress. The Genetics Program (as described in its report) has well-established multiple global collaborations that are ever expanding (in part due to a successful track record). By virtue of both being at the Broad Institute and having our Directors affiliated with MGH and MIT, we have many collaborative opportunities. As discussed at our August '07 Report/ Meeting, our past (2/07 to 2/09) funded research collaborations generated interesting research but most were not particularly mission-oriented. We still are using the '2-year model' (provide initial "seed funding" (over a maximum of two years) to allow the projects to obtain sufficient preliminary data either to qualify for other grants, or to determine if they are critical to Stanley Center goals. Past and current Stanley Center–supported collaborations are listed in Appendix D.

Finally, we should note that our Expenses (Appendix G) and Budget Appendix H) do not include the additional funds granted by SMRI specifically for patient collection. Although those funds are in the same MIT parent 'Stanley Center' account, we have assigned them to a separate sub-account to allow us to track them separately. Appendix A describes the details on both the progress of this collection effort and the budget plan. Expenses to date are minimal (~ $500,000) since the groups are still getting established; however, in Q1 '09, most staffing should be complete and recruitment rates on track, so we are arranging to have quarterly invoices. It is also worth noting that our actual expenses for Year 1 and expected expenses for Year 2 (ends 1/31/09) are both > 10% ($1 M) less than the funds granted and original budgets.

This is not surprising. It is due to a combination of ambitious plans for staffing in the first year, slower-than-expected renovations for the behavior lab, and delays in large-scale genetic analyses (both genotyping and sequencing) due to delays in receiving patient DNA samples and various unpredictable (but not unexpected) obstacles in developing NextGen Sequencing at the Broad Institute. Since our staffing will soon plateau (at ~50 to 55 FTEs), we already are accumulating large numbers of patient samples, and we have a large sequencing project beginning this month, we anticipate that 2009 will be quite productive.

In closing, we should also discuss the potential impact of the plans for the Broad Institute to establish itself as a permanent, non-profit organization, transitioning from its current form as an administrative unit within MIT. The endowment gift of the Broads is not supporting any research, but rather is dedicated to support the establishment and maintenance of the essential infrastructure to make and keep a permanent and independent entity. As the Broad Institute Director Eric Lander wrote in his Sept 4, 2008 announcement letter to the Broad community:

> *"Eli and Edythe Broad's extraordinary endowment gift, announced today, is not only a vote of confidence in what we have accomplished, but also a mandate to do even more and even better…The Broad endowment will allow the establishment of the Broad Institute as a permanent and unique entity within the scientific community. The Broad Institute will continue to have close collaborations among the partner institutions, focusing on the most critical projects in genomic biology and medicine."*

It is important to note that all the facilities and staff that currently comprise the Stanley Center will remain exactly the same. The capacity to undertake the scientific goals of this program will not change in any way. Additionally, this administrative change will have no impact on the collaborative nature of the Broad Institute or our scientific relationship with Harvard, MIT or the Harvard Hospitals.

We are excited about the potential opportunity. A transition team of has been working steadily to aim for a smooth transfer of most infrastructure and business systems effective July 1, 2009. The largest impact is on the administrative functions of Business Development, Contracts, Technology Licensing, Purchasing, Accounting, HR, IT, and Sponsored Programs. All contracts, including the SMRI grant that created the Stanley Center for Psychiatric Research and all subcontracts we have executed with collaborators, will be assigned from MIT to the new independent entity of the Broad Institute, effective July 1, 2009. The terms and conditions will be unchanged. Future contracts, Material Transfer Agreements, grant applications, etc. can now be handled directly by Broad staff, instead of requiring a second round of review and approval by MIT staff. Although we would be naïve to expect such a major transition without any bumps, it is unlikely that our research will be at all affected. Furthermore, we strongly believe that the new and significantly smaller (compared to MIT) structure will provide the Broad Community and the Stanley Center better communication and more control over administrative and business issues to allow effective and efficient operations. We look forward to the new discoveries we will make in 2009 and Year 3 of the Stanley Center.