X TUBEROUS SCLEROSIS COMPLEX RESEARCH PROGRAM

Tuberous sclerosis complex is a genetic disorder that can affect any or all systems of the body. The disorder is characterized by seizures, developmental delays, kidney disease, behavioral problems, and the growth of benign tumors (tubers) on vital organs such as the brain, kidneys, and heart. These tumors typically calcify with age, becoming hard (sclerotic). Children with tuberous sclerosis complex may have autistic-like symptoms. Tuberous sclerosis complex affects as many as 25,000 to 50,000 individuals in the United States and about 1 to 2 million individuals worldwide.[1] Although this disorder can be inherited as an autosomal dominant trait, two-thirds of cases are the result of a spontaneous genetic change on one of two genes, *TSC1* or *TSC2*. The *TSC1* gene is located on chromosome 9 and produces the protein hamartin. The *TSC2* gene is located on chromosome 16 and produces the protein tuberin. Hamartin and tuberin are believed to act as tumor growth suppressors. Therefore, their dysfunction may underlie the appearance of tumors that characterize tuberous sclerosis. There is currently no cure for this disease; however, surgical intervention and a number of treatments can help affected individuals.

Vision: To lessen the impact of tuberous sclerosis complex.

Mission: To encourage innovative research, including natural history studies, aimed at improved prevention, diagnosis, and treatment of tuberous sclerosis complex.

Congressional Appropriations for Peer Reviewed Research:

- $3M in FY02–03
- $3M in FY04
- $3.2M in FY05

Funding Summary:

- 7 awards from the FY02–03 appropriations
- 13 awards from the FY04 appropriation
- ~14 awards anticipated from the FY05 appropriation

PROGRAM BACKGROUND

The Department of Defense (DOD) Tuberous Sclerosis Complex Research Program (TSCRP) was established in fiscal year 2002 (FY02) by Joint Appropriations Conference Committee Report No. 107-350, which provided $1 million (M) for tuberous sclerosis complex research. The TSCRP has managed $9.2M through FY05 to fund peer reviewed tuberous sclerosis complex research. Twenty awards have been made through FY04 in an effort to advance the field of tuberous sclerosis research. Key initiatives of the TSCRP include support for groundbreaking concepts and ideas and the development of critical resources. Appendix B, Table B-7, summarizes the congressional appropriations and the investment strategy executed by the TSCRP for FY04 through FY05.

THE FISCAL YEAR 2004 PROGRAM

The TSCRP was continued through an FY04 congressional appropriation of $3M. Three award mechanisms were offered, one of which was previously established by the TSCRP (Idea Development Awards) and two that were new to the program in FY04 (Concept Awards

[1] *National Institute of Neurological Disorders and Stroke Fact Sheet, 2001;* Harrison's Principles of Internal Medicine, *15th Edition, McGraw-Hill, 2001.*

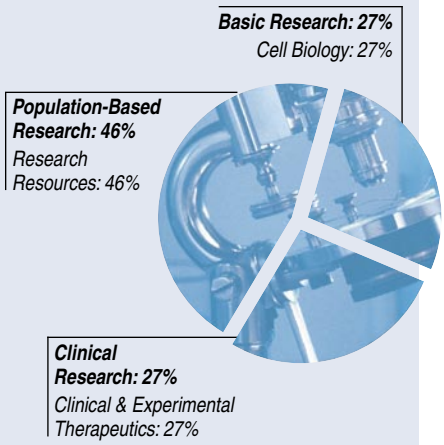


Figure X-1. FY04 TSCRP Portfolio by Research Area

"It was a privilege and honor to serve as Chair of the Tuberous Sclerosis Complex Research Program Integration Panel. Tuberous sclerosis complex is a devastating disease affecting children and adults, many of whom face the lifelong challenges of autism and mental retardation. During my 3 years of participation in the TSCRP peer-review system, it has been extremely rewarding to see innovative, state-of-the-art, clinically relevant research projects obtain funding. It was especially meaningful to work alongside consumer representatives whose families are directly impacted by tuberous sclerosis complex."

Elizabeth Henske, M.D., FY05 TSCRP Integration Panel Chair

and Natural History Development Awards). Of the 40 proposals received across award mechanisms, 13 were funded. The TSCRP's investment in Idea Development and Concept Awards was aimed at funding innovative, high-risk research relevant to tuberous sclerosis complex that resulted in 12 awards. (See the related box stories on pages X-5–X-6.) One Natural History Development Award proposal was funded to establish the development of multi-institutional natural history studies of tuberous sclerosis complex, including the establishment of the study teams, the preparation of clinical protocols and consent/assent forms, and the development of tools for data collection, analysis, and dissemination. FY04 Natural History Development awardees are required to submit a proposal and clinical protocol to the FY05 Natural History Study Award. Additional summary information about the number of proposals received, number of awards, and dollars invested for the FY04 TSCRP can be found in Table X-1. As illustrated in Figure X-1, the FY04 TSCRP has supported basic, clinical, and population-based research.

Table X-1. Funding Summary for the FY04 TSCRP

Categories and Award Mechanisms	Number of Proposals Received	Number of Awards	Investment
Research			
Concept	*26*	*7*	*$0.7M*
Idea Development	*13*	*5*	*$1.9M*
Research Resources			
Natural History Development	*1*	*1*	*$0.1M*
Total	*40*	*13*	*$2.7M*

THE VISION FOR THE FISCAL YEAR 2005 PROGRAM

Congress appropriated $3.2M to continue the TSCRP in FY05. The program retained the three award mechanisms offered in the previous FY (Concept, Idea Development, and Natural History Development Awards). In addition, the program launched the Natural History Study Award to fund focused, hypothesis-driven natural history studies to elucidate the clinical course of tuberous sclerosis complex. (Refer to the related box story on page X-8 for additional details about this mechanism.) A total of 60 proposals were received across award mechanisms, as shown in Table X-2, and approximately 14 awards are expected.

SCIENTIFIC OUTCOMES AND ADVANCES

While the first awards to TSCRP investigators were made in FY02, research funded by this new program is already producing results. The following projects represent some of the achievements made by TSCRP-supported investigators to lessen the impact of this genetic disorder.

Table X-2. Award Mechanisms Offered and Proposals Received for the FY05 TSCRP

Categories and Award Mechanisms	Number of Proposals Received
Research	
Concept	*42*
Idea Development	*17*
Research Resources	
Natural History Development	*0*
Natural History Study	*1*
Total	*60*

Dissecting the Molecular and Genetic Mechanisms of Learning Disabilities in TSC

Yi Zhong, Ph.D., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

The FY04 Concept Award mechanism, profiled in the box story on page X-5, provided funding for innovative basic and clinical research studies on the cutting edge of the tuberous sclerosis complex field. Dr. Yi Zhong of Cold Spring Harbor Laboratory received one of these awards to examine the contribution of TSC gene mutations to the development of learning disabilities, which are observed in a large number of individuals with tuberous sclerosis complex. His research utilizes the fruit fly *Drosophila melanogaster*, which shares similar molecular mechanisms of learning and memory with vertebrates and thus provides a powerful tool for analyzing the genetic basis of cognitive deficits. Dr. Zhong will assess the effects of inherited *tsc1* and *tsc2* genetic mutations on learning and determine whether expression of normal *tsc1* and *tsc2* genes in adult flies can correct the observed learning defects. He will also examine the consequences of enhanced expression of *tsc1* and *tsc2* in different regions of the brain during various stages of development and adulthood. Together, these studies should provide insight into whether learning deficits in tuberous sclerosis complex result from abnormal nervous system development or from the requirement for TSC1/TSC2 protein function in memory formation. Over 50% of tuberous sclerosis complex patients have learning disabilities, which are frequently associated with low self-esteem, poor self-image, and impaired social skills. Dr. Zhong's research into the molecular mechanisms underlying these deficits may ultimately aid in the development of new interventions that will improve the quality of life for these individuals and their families.

"It was an honor for me to serve as a consumer reviewer in the DOD's Congressionally Directed Tuberous Sclerosis Complex Research Program. The Peer Review process was extremely well organized and thorough. It brought together some the of country's leading scientists and clinicians to review and rate the grants that were submitted to the program. Even as a lay person, my views and input were considered equally with those of the experts. The army's inclusion of the consumer perspective helps insure that the program will meet the needs of those it is designed to serve. I came away feeling that I had made an enormous contribution to my community and felt privileged to be part of such a productive and effective process. I was also very appreciative to see such a talented panel coming together to help find the treatments that are needed for this devastating disease."

Celia Mastbaum, FY 02–04 Consumer Peer Review Panel Member

Fiscal Year 2005 Integration Panel Members

Elizabeth Henske, M.D. (Chair)
Fox Chase Cancer Center

Sandra Dabora, M.D., Ph.D. (Chair-Elect)
Brigham and Women's Hospital/ Harvard Medical School

Robert Finkelstein, Ph.D.
National Institute of Neurological Disorders and Stroke

Jackson Gibbs, Ph.D.
Merck Research Laboratories

Bruce Korf, M.D., Ph.D.
University of Alabama at Birmingham

Susan Lamont, Ph.D.
Tuberous Sclerosis Alliance

"I feel privileged to be involved as a consumer reviewer for the Tuberous Sclerosis Alliance and the CDMRP. The efficiency, the professionalism, the commitment, the constant conscious and conscientious effort to ensure that not one penny of our precious resources goes to waste are outstanding and must be commended. My son has struggled under the burden of tuberous sclerosis complex for all 12 years of his life. Perhaps through efforts like the CDMRP, someday he won't have to fight so hard. Thank you."

Patrick Sheffield, FY04–05 TSCRP Consumer Peer Review Panel Member

TSC1 Loss and Neurological Symptoms of Tuberous Sclerosis

Bernardo Sabatini, Ph.D., Harvard Medical School, Boston, Massachusetts

Most individuals with tuberous sclerosis complex are diagnosed as children and most have neurological symptoms, such as mental retardation or epilepsy. Although previous research has shown that tuberous sclerosis complex results from mutations in either the *TSC1* or *TSC2* genes, the molecular mechanisms underlying the observed neuropathology are not well understood. Dr. Bernardo Sabatini of Harvard Medical School, a recipient of an FY03 TSCRP Idea Development Award, has been studying whether the loss of normal *TSC1* gene expression disrupts the function of neurons in a cell-autonomous manner (i.e., only in the cells that do not express those genes). He prepared brain cell cultures from mice that carry a special form of the *TSC1* gene, known as a conditional allele. Expression of a modified form of a protein known as Cre recombinase resulted in loss of TSC1 protein expression in the cells. This loss of expression was associated with increased phosphorylation of the ribosomal protein S6, which indicates increased activity in the tuberous sclerosis complex signaling pathway. Dr. Sabatini also examined the effects of TSC1 loss on cell (primarily neuron) morphology. Cre recombinase was expressed in a small percentage of cells to create mixed, or mosaic, cultures in which a limited number of neurons lacking TSC1 were located in otherwise normal brain tissue. TSC1 loss resulted in increased neuron size, decreased dendritic spine density, and increased spine neck length and head size. This altered morphology may indicate perturbed neuron function. Preliminary experiments with rapamycin—which is undergoing preclinical and clinical testing for the treatment of tuberous sclerosis complex-associated tumors—showed that this drug abolished phosphorylation of the S6 protein in neurons that lack TSC1 and suppressed the effects of TSC1 loss on neuron size. However, rapamycin caused further disruptions in neuronal structure. Dr. Sabatini is continuing these experiments and his research is helping to shed light on the causes of the neurological symptoms seen in tuberous sclerosis complex. Ultimately, the knowledge gained from this study may be useful in the design and testing of therapies for those symptoms.

A CLOSER LOOK: THE FY04 CONCEPT AWARD

The Concept Awards were a highlight of the FY04 program. This award mechanism is patterned after the Breast Cancer Research Program's (BCRP's) Concept Award, which was launched in FY99 to fund the exploration of untested, high-risk questions that could give rise to testable hypotheses. The names of the submitting investigators, as well as their institutions, are removed from the proposals before review, ensuring that funding decisions are made based on the quality of the ideas and not the track record of the researcher or the reputation of the institution. As a result, this mechanism has attracted junior researchers as well as investigators from other research areas. Inspired by the successes of the BCRP mechanism, the TSCRP introduced Concept Awards in FY04, with each award providing a maximum of $100,000 for a 1-year period. A total of 26 Concept Award submissions were received, 7 of which received funding. The first recipients of the TSCRP Concept Award mechanism and a brief synopsis of their proposed research are included in the following list:

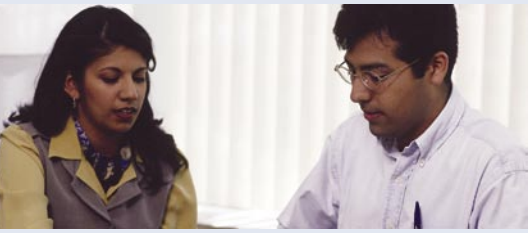
- Dr. Kun-Liang Guan of the University of Michigan will examine the regulation of the TSC1/TSC2 and Rheb proteins in cells and investigate how those proteins control cell growth. Improved understanding of the molecular and cellular mechanisms underlying tuberous sclerosis complex will help researchers devise better therapies for the disorder.
- Dr. David Sabatini of the Whitehead Institute for Biomedical Research plans to identify genes that regulate the growth of tuberous sclerosis complex but not normal cells. These genes may serve as excellent candidates for the development of tuberous sclerosis complex-specific drugs.
- Dr. Mustafa Sahin of Children's Hospital, Boston, seeks to use genetic screening to identify inhibitors of Rheb, a protein that is abnormally activated in tuberous sclerosis complex. The identified compounds may be used for the development of new tuberous sclerosis complex-specific treatments.
- Dr. Rachel Squillace of the Rothberg Institute for Childhood Diseases will develop cell lines from patients with lymphangioleiomyomatosis (LAM), a tuberous sclerosis complex-related disorder for which there are no approved therapies. These cell lines may be used for assessing the effectiveness of new treatments for LAM before they are tested in patients.
- Dr. Bo Xiao of Johns Hopkins University plans to create genetically engineered mice to examine the role of Rheb in the development of tuberous sclerosis complex symptoms in the brain and other organs. Moreover, these mice may also be useful for examining the effects of new tuberous sclerosis complex drugs in living organisms before human clinical trials.
- Dr. Li-Hui Xu of OncoImmune, Ltd., intends to assess the effectiveness of 2-deoxyglucose, which selectively kills *TSC1*/*TSC2* mutant cells in culture, in inhibiting tuberous sclerosis complex renal and brain tumor development in animal models. These studies will potentially provide important preclinical data that may serve as the foundation for future studies in tuberous sclerosis complex patients.
- Dr. Yi Zhong of Cold Spring Harbor Laboratory will use the fruit fly *Drosophila melanogaster* to study how mutations in the *tsc* genes affect learning and memory. These studies may help improve our understanding of the molecular events that give rise to learning disabilities and ultimately lead to new treatments for the cognitive deficits seen in over 50% of individuals with tuberous sclerosis complex.

The Concept Awards are being offered again in FY05 to promote the continued infusion of new investigators and ideas into the tuberous sclerosis complex field.

DISCOVERY AND DEVELOPMENT OF THERAPEUTICS FOR TUBEROUS SCLEROSIS COMPLEX

Researchers in the field of tuberous sclerosis complex are making great strides in our understanding of the basic molecular and cellular mechanisms underlying this disease. However, currently there is no cure for tuberous sclerosis complex and treatment options are solely targeted at treating the symptoms of the disease, not the molecular and cellular mechanisms underlying tuberous sclerosis complex. Thus, the progression of therapeutic agents and procedures based on the molecular causes of tuberous sclerosis complex remain a pressing need in the tuberous sclerosis complex community. The FY04 TSCRP offered Concept Awards to spark new and untested ideas relevant to tuberous sclerosis complex. Three of the seven funded FY04 Concept Award projects are aimed at just that—accelerating the progression of novel therapeutics for tuberous sclerosis complex based on the molecular and cellular basis of the disease. The following exciting projects are profiled in this section and represent the collective effort of researchers working to lessen the impact of tuberous sclerosis complex:

- Study of 2-Deoxyglucose as a Potential Treatment for Tuberous Sclerosis Complex
- Identifying Novel Drug Targets for the Treatment of Tuberous Sclerosis Complex Using High-Throughput Technologies
- Development of Peptide Inhibitors of Rheb Signaling Pathway



Study of 2-Deoxyglucose as a Potential Treatment for Tuberous Sclerosis Complex

Li-Hui Xu, M.D., Ph.D., MBA, OncoImmune, Ltd., Columbus, Ohio

Recent studies have indicated that cells carrying mutations in *TSC1* or *TSC2* (the genes responsible for the tuberous sclerosis complex) are very sensitive to cellular energy starvation. Glucose deprivation in these cells results in a significant increase in cell death. 2-Deoxyglucose (2-DG) is a non-metabolized analog of glucose that has been recently shown to induce cell death only in cells carrying a mutation in *TSC1* or *TSC2* without affecting normal cells. 2-DG has been shown to cross the blood–brain barrier, a significant finding since the majority of patients with TSC develop brain disorders. Thus, these results suggest that 2-DG may be an exciting therapeutic agent for the treatment of tuberous sclerosis complex. TSCRP-supported investigator Dr. Li-Hui Xu will obtain important preclinical data in an animal model to determine whether 2-DG can inhibit tumor growth caused by *TSC1* or *TSC2* mutations.

Identifying Novel Drug Targets for the Treatment of Tuberous Sclerosis Complex Using High-Throughput Technologies

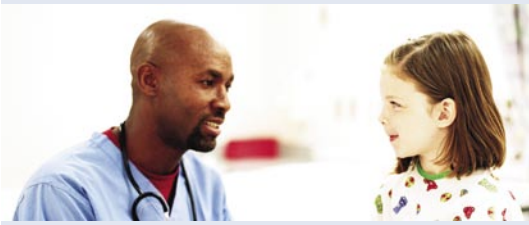
David Sabatini, M.D., Ph.D., Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

A patient with tuberous sclerosis complex has mutations in one of two genes, *TSC1* or *TSC2*. The protein products of these genes are involved in controlling cell growth and are believed to act as tumor growth suppressors. However, little is known about the signaling pathways and proteins involved in this response. TSCRP-funded investigator Dr. David Sabatini believes that if we could identify genes that, when their expression is decreased by RNA interference (a technique by which the expression of genes can be silenced), cause only those cells carrying mutations in *TSC1* or *TSC2* to stop growing, die, or revert to normal, patients with tuberous sclerosis complex could be successfully treated. Using a technique called high-throughput screening, Dr. Sabatini plans on testing every gene in the genome whose expression is decreased by RNA interference for their ability to prevent the growth, induce cell death, or cause a reversion to normal of only those cells with a mutation in *TSC1* or *TSC2*. These genes would then be excellent candidates for small molecule drug development.

Development of Peptide Inhibitors of Rheb Signaling Pathway

Mustafa Sahin, M.D., Ph.D., Children's Hospital, Boston, Massachusetts

Tuberous sclerosis complex is caused by mutations in either the *TSC1* or *TSC2* gene. The protein products of *TSC1* and *TSC2* are hamartin and tuberin, respectively. Previous studies have shown that hamartin and tuberin can form a complex that can negatively regulate the small G protein, Rheb. Rheb is an activator of mammalian target of rapamycin (mTOR), a protein kinase involved in the regulation of cell size and proliferation. Studies have shown that in the absence of functional hamartin or tuberin, levels of active Rheb increase, which in turn leads to an increase in cell size and proliferation and eventually tuberous sclerosis complex. TSCRP-funded researcher Dr. Mustafa Sahin proposes to develop peptide inhibitors of Rheb using a genetic screen strategy. These inhibitors can then be used to provide important insights into the cellular functions of Rheb as well as the development of novel therapeutics for the treatment of tuberous sclerosis complex.



"The CDMRP program for tuberous sclerosis complex has been a tremendous benefit to research progress in multiple aspects of this disease whose genes are at the crossroads of multiple signaling pathways. In the 3 short years since the inception of the TSCRP, research proposals have come from nearly 100 different labs, including both leading established scientists and those at the beginning of their research careers, and including studies on a variety of model organisms, analysis of interacting genes and proteins, and discovery or development of novel therapeutics. The Concept Award format has in particular stimulated much thought and interest in the research community, leading to submission of many exciting and novel proposals, which otherwise would not benefit from funding."

David J. Kwiatkowski, M.D., Ph.D., FY02–05 TSCRP Scientific Peer Review Panel Member

A CLOSER LOOK: THE FY05 NATURAL HISTORY STUDY AWARD

Last year's annual report featured a box story on the FY04 Natural History Development Award (NHDA), which was created in response to the need of the tuberous sclerosis complex community for rigorous natural history studies. Such studies would complement ongoing laboratory and basic research initiatives by characterizing tumor growth and other disease manifestations in affected individuals, as well as analyzing correlations between specific *TSC* gene mutations and symptoms. Lack of data on the natural history of tuberous sclerosis complex has hampered efforts to predict disease prognosis and evaluate the effectiveness of new therapies in clinical trials. The NHDA addressed this need by funding the development of key resources necessary for submission of a complete natural history study proposal to the FY05 Natural History Study Award mechanism. This new mechanism supports focused projects that test specific hypotheses or answer clearly defined questions regarding the natural history of tuberous sclerosis complex. FY04 NHDA recipients are required to prepare and submit Natural History Study Award proposals in FY05 as a product of their initial funding. However, all eligible applicants with tuberous sclerosis complex-relevant natural history studies may compete for this award, which provides up to $1M over 3 years. Applicants must explain how their research would improve the clinical management of the disease or aid in the testing of novel therapeutics. All proposals must also include a clinical protocol and comprehensive plans for participant recruitment, study management, data analysis, and public dissemination of study results. Funding decisions for the FY05 Natural History Study Award are scheduled to be made in mid- to late 2005. This award mechanism, by supporting exceptional investigators in their efforts to characterize the clinical course of TSC and improve the management and treatment of the disease, is bringing the field one step closer to achieving the TSCRP's goal of enhancing the quality of life for individuals with tuberous sclerosis complex and their families.

Signs and Symptoms

Because tuberous sclerosis complex affects multiple organs, a variety of symptoms may be experienced. The disorder can cause benign tumors, called tubers, to grow in various organs, including the skin, brain, heart, kidneys, lungs, and eyes. However, in most individuals with tuberous sclerosis complex, only some of these organs are involved, and symptoms vary depending on which organs and systems are affected. In addition to the growth of benign tumors, other signs and symptoms of tuberous sclerosis complex include seizures, mental disabilities, skin abnormalities, and behavior problems. Some patients with tuberous sclerosis complex also develop renal cell carcinoma and other malignant tumors.

BOTTOM LINE

Since FY02, the DOD TSCRP has been responsible for managing $9.2M in congressional appropriations, resulting in 20 awards through FY04. Projects funded by this newly established program are anticipated to lead to the substantial improvement in the understanding, diagnosis, and treatment of tuberous sclerosis complex and enhance the quality of life of persons with the disease. Research highlights, award data, and abstracts of funded TSCRP proposals can be viewed on the Congressionally Directed Medical Research Programs (CDMRP) website (http://cdmrp.army.mil).