VII. Tuberous Sclerosis Complex Research Program

"There is a stereotype that government programs are bureaucratic and lack efficiency and accountability; however, the CDMRP proves that image wrong on all counts. I have never participated in a more thoughtfully designed or intelligently executed program. Although I have served on a variety of boards and committees, I have never seen one more inviting of feedback or more serious about improving each detail of the experience. As a result, every piece in place is efficient and effective, and the whole program really works to fulfill the mission of the CDMRP. As a consumer reviewer for the Tuberous Sclerosis Medical Research Program, I was thrilled to bring back firsthand news to my local community of the first-rate work you are doing. The program is a model, and it was an honor and an inspiration to participate."

Laura Jenson
TS Alliance
FY06 Peer Reviewer

Generating Momentum

Vision

To lessen the impact of tuberous sclerosis complex.

Mission

To encourage innovative research aimed at understanding the pathogenesis of TSC and improving its diagnosis and treatment.

The Disease

Tuberous sclerosis complex (TSC) is a genetic disorder that can affect any or all systems of the body.

- TSC affects as many as 50,000 individuals in the United States and about 1 to 2 million individuals worldwide.
- Although this disorder can be inherited as an autosomal dominant trait, two-thirds of the 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.

Signs and Symptoms

Because TSC affects multiple organs, a variety of symptoms may be experienced. The disorder can cause benign tumors, called hamartomas, to grow in various organs, including the brain, skin, heart, kidneys, lungs, and eyes. However, in most individuals with TSC, only some of these organs are involved, and symptoms vary depending on which organs and systems are affected.

Other signs and symptoms of TSC include:

- seizures
- mental disabilities
- skin abnormalities
- behavioral problems
- brain tumors
- autism
- kidney disease
- lung complications
- developmental delays

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 TSC research. The TSCRP has managed $17.5M through FY08 to fund peer-reviewed TSC research, as shown in Figure VII-1. A total of 48 awards have been made through FY06 in an effort to advance progress in the field of TSC research.

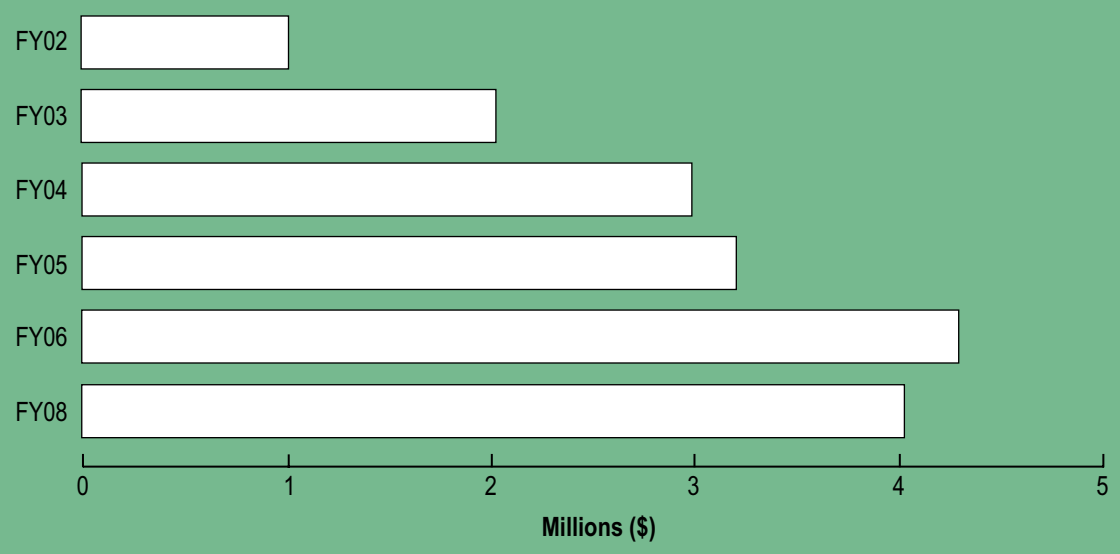

Figure VII-1. TSCRP Appropriations

The TSCRP returned in FY08 after a 1-year lapse in funding in FY07. During this time, TSC research progressed with the continuation of discoveries from previously funded awards. TSC researchers generated significant findings through the use of existing research resources and model systems. They also accelerated the research by linking TSC proteins with vital cellular signaling pathways in other diseases. The TSCRP continued to monitor the progress of awards and reported important findings to the community through highlights on the Congressionally Directed Medical Research Programs (CDMRP) website and meetings with other TSC funding agencies. New funding mechanisms were created in FY08 by the TSCRP to overcome roadblocks in TSC research.

"The collaboration between patients and their families, grant reviewers, and extremely high-quality research proposals resulted in the selection of outstanding proposals that will accelerate the understanding of Tuberous Sclerosis Complex and the development of effective targeted therapy. I was honored to serve as a grant reviewer in the Congressionally Directed Medical Research Programs and know that because of this program, my patients will be helped."

John J. Bissler, M.D.
Cincinnati Children's Hospital Medical Center
FY04-FY05 Peer Reviewer

The Best People

The TSCRP has integrated the best people in all aspects of program execution. Consumer advocates, peer review panel members, Integration Panel (IP) members, and the scientific community have worked synergistically to achieve the goal of lessening the impact of TSC. The combination of their diverse expertise has generated ideas that have hastened TSC research.

Consumer Advocates

Consumer advocates for the TSCRP may be individuals with TSC or those who have family members with TSC (TSC initially manifests in childhood). Their firsthand experiences with TSC provide a unique perspective that helps scientists understand the human side of the disease and encourages funding decisions that reflect the concerns and needs of patients, patients' families, and clinicians. Consumer advocates also share what they have learned with their communities, resulting in increased awareness of the importance of research and a stronger relationship between the scientific and consumer advocate communities. Consumer advocates participate in program development and execution by deliberating the issues and concerns unique to the TSCRP, developing an annual investment strategy, and participating in both peer and programmatic review of proposals. Further details about consumer participation can be found in Section I, Overview.

Peer Review Panel Members

Scientific peer review is a criterion-based process where proposals submitted to the TSCRP are evaluated on scientific and technical merit. Peer review panels are typically organized by scientific discipline and specialty area and are composed of prominent TSC-focused scientists and clinicians and dedicated consumer advocates. Scientific reviewers are selected for their subject matter expertise and experience with scientific peer review. Consumer reviewers are nominated by an advocacy or support organization and are chosen on the basis of their leadership skills and their commitment to advocacy, support, and outreach, as well as their interest in expanding their scientific knowledge. More than 65 scientists, clinicians, and consumer advocates have contributed their expertise to peer review for the TSCRP. Additional details about peer review can be found in Section I, Overview.

"Once an orphan disease, TSC has become a prime example of how bench-to-bedside research is offering new treatments and hope to the affected individuals and their families. The progress that we have witnessed over the last 6–7 years in TSC research is a testament to the amazing dedication of a group of scientists who have benefited directly from the funding opportunities such as those offered through the TSCRP. My interaction with the people involved with the Integration Panel reaffirms the strong committment of the DOD toward their mission to improve the health of those suffering from TSC and related disorders."

Raymond Yeung, M.D.
University of Washington
FY08 TSCRP IP Chair

Integration Panel Members

The TSCRP IP is composed of a diverse group of distinguished scientists, clinicians, and consumer advocates. Individual panel members recommend the program vision and investment strategy and participate in the programmatic review of research proposals. IP members have dedicated significant hours in support of the TSCRP, and their commitment, passion, and intellect have driven progress in the field.

Scientific Community

The TSCRP has funded 42 scientists and clinicians across the nation and abroad. TSCRP-supported investigators are engaged in cutting-edge work to find a cure for TSC. Through creative approaches and ideas, investigators are gaining momentum toward the goal of lessening the impact of TSC, as reflected in the success stories described in the remainder of this chapter.

FY08 TSCRP IP Members

Raymond Yeung, M.D. (Chair)
University of Washington

Sandra Dabora, M.D., Ph.D.
(Chair Emeritus)
Brigham and Women's Hospital

Jane Fountain, Ph.D.
National Institute of Neurological Disorders and Stroke

Susan Lamont, Ph.D.
Tuberous Sclerosis Alliance

E. Steve Roach, M.D.
The Ohio State University

Sabita Sankar, Ph.D.
Signal Pharmaceuticals LLC, Division of Celgene Corporation

Elizabeth Thiele, M.D., Ph.D.
Massachusetts General Hospital

Tian Xu, Ph.D.
Yale University School of Medicine

Generating Momentum

Through Teamwork

Building on Research Resources

The TSCRP implemented a research resources initiative in 2007 to publicize newly available resources supported by the TSCRP. The goal of this initiative is to encourage collaboration within the TSC research community as well as resource sharing by making information publicly available. The reference table of research resources can be accessed at http://cdmrp.army.mil/tscrp/tscresources.htm.

To date, 26 resources have been posted. Several of the Principal Investigators have been contacted with requests for specific resources. Additionally, some of the resources were used in scientific publications. Examples include:

- TSC natural history database (Sparagana, Steven and Whittemore, Vicky): Researchers have asked about access to the data, participation in the project, and advice on developing a database
- TSC yeast strains (Henske, Elizabeth): Request for TSC1-null and TSC2-null strains
- TSC *Drosophila* model (Su, Tin Tin): Request for homozygous TSC1 mutant *Drosophila* larva model and assay for screening drugs
- Human lymphangioleiomyomatosis (LAM) primary cultures (Castro, Ariel)
- Database containing messenger RNAs that are translationally regulated by sera and rapamycin in a TSC1/TSC2-mediated manner (Stokoe, David): Publication in *Molecular and Cellular Biology*

Sharing Ideas with Other Funders

The TSCRP again participated in a yearly meeting with other agencies and organizations that fund TSC research, including the TS Alliance and National Institutes of Health. Representatives from these groups met to discuss progress in TSC research, current TSC grants, and collaborative initiatives between funding agencies. The TSCRP also participated in a major TSC scientific conference sponsored by the TS Alliance and an mTOR [mammalian target of rapamycin] signaling conference sponsored by the National Institute of Neurological Disorders and Stroke. These meetings helped inform the TSCRP of the most recent important discoveries and research directions.

Generating Momentum Through Scientific Discoveries

Using Diverse Model Systems

Using *Drosophila* to Learn How TSC Affects the Timing of Neuronal Differentiation

Helen McNeill, Ph.D.
Mount Sinai Hospital, Toronto

Dr. Helen McNeill used *Drosophila* model systems to discover how TSC loss affects the timing of neuronal differentiation. With funding from an FY05 Idea Development Award, Dr. McNeill's group used TSC2 knockout flies as well as flies that were transgenic for Pointed P2 (PntP2), a transcription factor that is regulated by TSC1/2. The team also used Drosophila cell lines with knocked down TSC1 expression levels.

Dr. McNeill used in vivo analysis to show that loss of TSC led to increases in the levels of PntP2, a transcription factor necessary for neuronal differentiation. In addition, the group has identified several novel factors that are regulated by the InR/TSC pathway and that regulate the timing of neuronal differentiation. These may provide novel targets for clinical treatments. In addition, their genetic analysis revealed that loss of S6K blocked the process of precocious neuronal differentiation in *Drosophila* that lacked functional TSC1/2. S6K is a key mediator of TSC1/2-mTOR-mediated signaling and a candidate therapeutic target for treating TSC. These findings may lead to the development of novel, early clinical interventions for individuals with TSC to prevent or ameliorate the nervous system defects associated with this disease.

Using Zebrafish to Understand Kidney and Neural Disease Manifestations of TSC

Zhaoxia Sun, Ph.D.
Yale University

Dr. Zhaoxia Sun and her colleagues are using a zebrafish model to study kidney development and the formation of cysts in the absence of functional TSC1. Kidney cysts can occur in TSC patients, and the cell surface organelle and cili play a central role in kidney cyst formation. This finding led Dr. Sun's team to hypothesize that there is a functional connection between TSC1 and the cilium. Using funding from an FY06 Concept Award, the researchers knocked down TSC1 and demonstrated a cystic kidney phenotype. They are currently exploring the molecular and cellular mechanisms causing this phenotype. Results from this study could lead to new therapeutic targets for treating kidney cysts in TSC patients.

Scott Baraban, Ph.D.
University of California, San Francisco

Dr. Scott Baraban is using a zebrafish model to study neural effects resulting from loss of TSC1. Dr. Baraban's team is determining whether loss of TSC gene function, in the absence of tuber formation, results in seizures. This is a fundamental question for TSC research because it is unclear whether there is a causative, correlative, or nonexistent relationship between tubers and seizures. Furthermore, seizures affect approximately 90 percent of TSC patients. Using funding from an FY05 Concept Award, the team is currently analyzing seizure-like behavior in TSC1-deficient zebrafish and exploring the effects of rapamycin on these animals.

Using Fission Yeast to Dissect the Molecular and Cellular Role of Rheb

Fuyuhiko Tamanoi, Ph.D.
University of California, Los Angeles

Dr. Fuyuhiko Tamanoi used a fission yeast model system to determine the role of Rheb in cellular pathways. Rheb is a member of the Ras superfamily, is expressed at high levels in the brain, bridges the TSC1/TSC2 and mTOR proteins, and is essential for growth of fission yeast.

Using funding from an FY04 Idea Development Award, Dr. Tamanoi's team discovered 22 amino acid changes in fission yeast mTOR (Tor2p) that bypassed the requirement of fission yeast Rheb (Rhb1p) for cell growth. These mutations clustered in highly conserved residues in the FAT and kinase domains, suggesting an important function in the human mTOR protein. Further studies with these mutant cells revealed that Rhb1p was required for resistance to conditions of stress such as high salt, high temperature, and toxic amino acid analogs in fission yeast. These results could lead to a smarter design of Rheb inhibitors, such as farnesyltransferase inhibitors, which may be used to reverse phenotypes associated with TSC deficiency.

Linking TSC Proteins to Key Signaling Pathways

Linking TSC1/2 and p53

Kun-Liang Guan, Ph.D.
University of California, San Diego

Dr. Kun-Liang Guan established a vital link between TSC1/2 and the p53 tumor suppressor gene. Using funding from an FY05 Concept Award, Dr. Guan's team showed that constitutive mTOR activity amplified p53 activation, in vitro and in vivo, by stimulating p53 translation. The inactivation of mTOR prevented cell death by nutrient stress and genomic damage via p53. However, loss of TSC1 or TSC2 and consequential activation of mTOR resulted in dramatic accumulation of p53 and apoptosis in response to stress conditions. The team showed that p53 levels were elevated in TSC tumors, which rarely become malignant. The researchers concluded that the coordinated relationship between mTOR and p53 during cellular stress provides a possible explanation for the benign nature of hamartoma syndromes, including TSC. Additionally, the findings have clinical significance, as they suggest that the efficacy of mTOR inhibitors in antineoplastic therapy may also depend on p53 status, and mTOR inhibitors may antagonize the effects of genotoxic chemotherapeutics.

Linking FK-506 Binding Proteins (FKBP) to TSC

Yongjian Liu, M.D., Ph.D.
University of Pittsburgh

Dr. Yongjian Liu and his co-investigator, Dr. Yu Jiang, discovered that Rheb regulates mTOR through FKBP38. FKBP38 plays important, diverse roles in mediating neuronal apoptosis; anchoring the 26S proteasome to the mitochondria; trafficking the HERG protein (which causes the cardiac disorder Long QT Syndrome); mediating TSC1/2-dependent cell size regulation; and aiding neuroprotection in neurodegenerative diseases. Using funding from an FY05 Concept Award, Dr. Liu's team showed that Rheb interacted directly with FKBP38 and prevented its association with mTOR in a GTP-dependent manner. Overexpression of FKBP38 prevented insulin-stimulated phosphorylation of the mTOR targets S6K, S6, and 4E-BP1. The researchers found that FKBP38 was an endogenous inhibitor of mTOR for which inhibitory activity was antagonized by Rheb in response to growth factor stimulation and nutrient availability. This finding suggests that FKBP38 may be a new therapeutic target for treating TSC.

Combining Techniques for Comprehensive Neurological Analyses

Peter Crino, M.D., Ph.D.
University of Pennsylvania

Dr. Peter Crino, an FY05 Idea Development Award recipient, provided the first comprehensive molecular-anatomic view of a neurodevelopmental disorder associated with epilepsy and autism through analysis of postmortem brains from TSC patients. Dr. Crino's group used a unique approach that combined postmortem magnetic resonance imaging (MRI), histology, and immunocytochemical analysis. The researchers discovered subtle cytoarchitectural abnormalities that were undetectable by MRI alone. The findings suggest that for many TSC patients, structural lesions in the brain are widespread and pervasive and may explain why many individuals with TSC suffer from severe epilepsy or autism when only a solitary tuber or brain lesion is observed using MRI. This study may lead to new strategies of early or in utero treatment with mTOR inhibitors such as rapamycin to prevent the effects of TSC gene mutations on brain formation.

Generating Momentum Through Creative New Funding Mechanisms

In FY08, the TSCRP IP designed two new award mechanisms to overcome roadblocks in TSC research and generate additional momentum in the field. The Clinical Research Award supports TSC research projects performed by clinical researchers involved in the support of clinical care of individuals with TSC. As basic science discoveries in TSC continue to increase, translating these findings to patients is becoming increasingly important. The Clinical Research Award could help bridge the gap between "bench and bedside."

The Career Transition Award supports TSC researchers during the transition from postdoctoral training to an independent position. This award has the unique feature of funding up to 2 years of postdoctoral training followed by up to 2 years of a faculty-level position. The Career Transition Award addresses the important issue of retaining young scientists in TSC research and helps maintain the momentum and expansion of discoveries from a postdoctoral project into a new, independent TSC research laboratory.

The Program Today

The Vision for FY08

Although the TSCRP did not receive an appropriation in FY07, Congress appropriated $4M in FY08 to continue the program. The TSCRP requested proposals in 4 award mechanisms in support of clinical research, innovative research, and training/recruitment. Highlights of each award mechanism are described as follows:

- Clinical Research Awards represent an original new award mechanism to the TSCRP to support TSC research projects performed by clinical investigators involved in the clinical care of individuals with TSC. Projects should lead to a demonstrated improvement in clinical outcome or the development of a metric for clinical outcome.
- Concept Awards support the exploration of untested ideas relevant to TSC and/or the development of novel preclinical tools needed to advance TSC research.
- Idea Development Awards encourage innovative research directed toward the pathogenesis of TSC and improving its diagnosis and treatment.
- Career Transition Awards also represent a new award mechanism to the TSCRP to support TSC researchers during the transition from postdoctoral training to independent positions.

A total of 55 proposals were received across award mechanisms, as shown in Table VII-1, and approximately 8 awards are anticipated. Appendix B, Table B-6, summarizes the congressional appropriation and the investment strategy executed by the TSCRP for FY08.

Table VII-1. Award Mechanisms Offered and Proposals Received for the FY08 TSCRP

Categories and Award Mechanisms	Proposals Received
Clinical Research	
Clinical Research	2
Innovative Research	
Concept	24
Idea Development	27
Training/Recruitment	
Career Transition	2
TOTAL	**55**

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