

## **Tuberous Sclerosis Complex: From Genes to New Therapeutics**

### **1. Conference Summary**

On September 23-25, 2007, 185 conference participants met at the Loews Annapolis Hotel in Annapolis, MD for an international conference on tuberous sclerosis complex (TSC). The main goals of the conference were to provide an update on the status of TSC research and update the National Institutes of Health TSC Research Plan (originally written following the 2002 TSC Conference in Chantilly, VA). Individuals from Canada, England, Germany, India, Italy, Japan, The Netherlands, Norway and the U.S. attended the 2 ½ day conference. In addition, 17 TSC Clinic Directors and 10 TSC Clinic Coordinators arrived early for a ½ day TSC Clinic meeting prior to the conference.

Sponsors for the conference include: ORD, NINDS, NIAMS, NIMH, NCI and NIDDK, National Institutes of Health Conference Grant to the TS Alliance (Principal Investigator: Vicky Whittemore), The Rothberg Institute, Sandra & Brian O'Brien, Novartis Oncology, Joy & Chris Dinsdale, Wyeth, and UCB Pharma.

Each of the sessions started with a parent of an individual with TSC talking about living with TSC and the impact on the individual and on the family. Many of the conference participants commented that these presentations were very meaningful and moving. Each session was followed by a breakout discussion that focused on identifying the critical questions that need to be addressed in that area of TSC research and the gaps in research.

### **Opening Session**

The Opening Session started with a welcome from Kari Luther Carlson, Acting CEO and Terry Elling, Chair, TS Alliance Board of Directors. Overview lectures on the basic science of TSC by Dr. Cheryl Walker and the clinical manifestations of the disease by Dr. Michael Frost set the stage for the clinicians who are not familiar with the TSC1/2-mTOR signaling pathway, and for basic scientists who are not aware of the various manifestations of TSC.

### **Session I: Regulation of Growth and Neoplasia in TSC and LAM**

Session I provided an update on the most recent findings related to the role of the TSC1/2 complex in the mTOR signaling pathway, new potential targets for drug development, and the potential role of the mTOR pathway in other diseases such as polycystic kidney disease (PKD). Dr. Elizabeth Petri Henske described her laboratory's attempt to identify the cell of origin that causes angiomyolipomas and the lesions found in the lung in lymphangiomyomatosis (LAM) using the fruit fly (*Drosophila*). She also described an animal model they are developing to address the question of estrogen's role in LAM. Drs. Shaw, Hengstchlager, Fielhaber, Krymskaya, Simon and Brugarolas all described various functions of the TSC genes in cell growth, the cell cycle, cell migration and in response to hypoxia (lack of oxygen). Dr. Thomas Weimbs talked about the ongoing research in his laboratory studying the role of the polycystin-1 (PC-1) gene (the gene for autosomal dominant polycystic kidney disease (ADPKD) that is located next to the TSC2 gene on chromosome 16). PC-1 has recently been shown to regulate the mTOR and STAT 6 pathways, and animal models of ADPKD respond to mTOR inhibitors (rapamycin).

### **Session II: Signaling Defects in the mTOR Pathway and TSC**

Session II continued the discussion of the signaling defects in the mTOR pathway in TSC, and the role of angiogenesis in TSC skin lesions and other tumors. Dr. Tom Darling described his work using cells derived from facial angiofibromas to study the role of the TSC genes in skin lesions and the role of angiogenesis in the lesions. He described his laboratory's research using both cells grown in tissue culture dishes and animal models to

study skin lesions in TSC. Drs. Guan, Lamb, Manning, Robb and Duevel all described various aspects of the mTOR pathway and the role of the TSC genes, and Dr. Sabatini discussed a new animal model in which the gene for Rheb was knocked out, and asked for input from the participants on the interesting abdominal lesions they have observed in this model.

### **Session III: Epileptogenesis and Neuropathology in TSC: Brain Development, Cell Growth and Migration**

Dr. Peter Crino described his laboratory's approaches to study three questions:

1. How does loss of function of TSC1 or TSC2 in cells that will become either neurons or glia in the brain lead to altered cell morphology and the brain lesions seen in TSC? They have utilized TSC brain specimens to look for mutations in the cells in tubers and found evidence of two hits in some of the cells in the tubers. They also found more widespread pathology in the brains of individuals with TSC, and speculate that brain dysfunction in TSC is a result of more widespread abnormalities that are not seen using brain imaging (MRI).
2. What is the embryonic lineage (where do they come from?) of the cells in TSC brain lesions? Their results and previous studies indicate that cells in tubers, subependymal nodules and subependymal giant cell tumors result from a common embryonic progenitor cell in the subventricular zone.
3. How does altered brain development lead to epilepsy and cognitive disabilities in TSC? Their studies indicate that there is a relative excess of excitatory cells and a reduction in inhibitory cells in tubers that may relate to epileptogenesis.

Dr. Khababullin described their research utilizing *Drosophila* (fruit flies) in which they found abnormal cell differentiation when the TSC1 gene was mutated. Tristan Sands described the mouse model they developed in which the TSC1 or TSC2 gene is mutated in embryos in utero, resulting in neuronal migration defects. Dr. Ess discussed the mouse model he has developed in which they turned off the TSC1 gene in neural progenitor cells at very early stages of brain development. They are currently characterizing these animals, and studying the abnormal cells in the cerebral cortex of the animals. Dr. Wong discussed their ongoing studies utilizing another mouse model in which the TSC1 gene is knocked out primarily in glial cells in the brain, resulting in animals with abnormal brain development and epilepsy. Dr. Jensen described the work that has been ongoing in her laboratory characterizing the abnormal cells in tubers from human brain specimens, as well as work with animal models of TSC to investigate the cause of epilepsy in these animals. Her studies point to abnormal receptors for the excitatory neurotransmitter, glutamate. Dr. Wu discussed the use of magnetoencephalography, FDG-PET/MRI co-registration and diffusion-tensor imaging (DTI) in children with TSC to identify the epileptogenic focus.

### **Session IV: Neurocognition in TSC: Neuronal Development, Synaptogenesis and Behavioral Phenotypes in TSC**

Dr. Petrus de Vries started the session with a discussion of a theory he has developed that he calls Global Regulator and Integrator of a Range of Physiological Processes (GRIPP). Their hypothesis is that structural brain abnormalities and/or seizures are neither necessary nor sufficient to explain the learning and behavioral profiles observed in individuals with TSC. Under GRIPP, the spectrum of neurodevelopmental abnormalities is caused by the wide-ranging functional consequences, at a protein level, of different TSC1 or TSC2 mutations.

Dr. Mark Zervas described elegant studies using genetic techniques to study neuronal development in mice that could be applied to the study of brain development in the animal models of TSC. Dr. Michael Gambello described a new animal model that he has developed

that specifically targets radial glia cells – cells that are critical in the development of the brain. Dr. Joseph Bateman described their ongoing research in *Drosophila* (fruit fly) to study patterns of neuronal differentiation and development. Dr. Linda van Aelst described their research on the genes involved in appropriate neuronal development and polarity (essentially the appropriate development of a north and south for neurons in the brain). Dr. Veronica Alvarez discussed their research specifically focused on the dendrites of neurons and the role of the TSC genes in the formation and function of these processes of neurons in the brain. Dr. Mustafa Sahin described abnormal formation of connections in the TSC2 knockout mice, and their planned studies to investigate the link between TSC, epilepsy and autism. Dr. Diane Chugani discussed the role of the mTOR pathway in autism in several different disorders (TSC, Rett Syndrome, Fragile X, etc.) and their ongoing imaging studies related to TSC and autism. Deborah McCartney described visuospatial processing problems in individuals with TSC, including unilateral neglect in which individuals favored information on one side of the screen over the other (similar to what is seen in individuals following a stroke). Dr. Elizabeth Thiele discussed the data they are collecting on psychiatric issues in adults with TSC, especially depression, anxiety and socialization issues.

### **Session V: Clinical Manifestations and Therapeutic Opportunities in TSC**

Dr. David Kwiatkowski provided a brief history of TSC from the identification of the genes, the discovery of the role of the TSC1/2 complex in the mTOR signaling pathway, animal models that are currently being utilized to study this pathway and recent studies in the mouse models testing potential new drugs to regulate the mTOR pathway. Dr. Rosemary Ekong gave an overview and update of the TS Alliance-funded TSC Variation Databases that now have cataloged over 2275 entries of variants. Jane Cox described the data collected in the Renal Registry in the U.K. and Dr. Susana Camposano discussed the efficacy of vigabatrin to treat infantile spasms in children with TSC.

Dr. Sandra Dabora discussed the research in her laboratory that led to the current multicenter clinical trial to test the efficacy of rapamycin (Sirolimus) to treat renal angiomyolipomas. At the time of the conference, there were 25 individuals enrolled in the study, and she reported their preliminary results that there are 4 responders. Dr. Julian Sampson reported on the ongoing trial in the U.K. with rapamycin, which will be a two-year study in which they are also performing neuropsychological evaluations of all of the study participants. Dr. David Franz discussed the results of the initial single site study with rapamycin (now in press), and their current studies using RAD001 (Everolimus) to study the efficacy of this drug to treat renal angiomyolipomas and subependymal giant cell tumors.

## **2. Planned Publications**

A summary of the conference will be posted on the TS Alliance web site and published in the quarterly magazine, *Perspective*. Several of the speakers at this conference have been invited to author chapters in the fourth edition of the book *Tuberous Sclerosis Complex* that will incorporate the discussions held during the conference. In addition, the TS Alliance is finalizing a TSC Research Strategic Plan that will outline the focus of the TS Alliance research initiatives for the next five years.

## **3. Proposed or Effected Research Activities for Rare Diseases**

The Tuberous Sclerosis Alliance released Requests for Applications for Letters of Intent for several grant mechanisms in response to the discussions held at the conference. The RFAs were written to solicit research in those areas identified to be gaps in TSC research and to answer the essential questions regarding both basic and clinical aspects of the disease that will then lead to the development and testing of new treatments and therapies for TSC. IN

addition, the TSC Clinic Directors discussed the need to identify funding for the TSC Clinical Network in order to provide support for the infrastructure of the TSC Clinics throughout the U.S. as they continue to expand in the clinical care of individuals with TSC from children through adults, participate in clinical trials, participate in the TSC Natural History Database project and other research efforts.