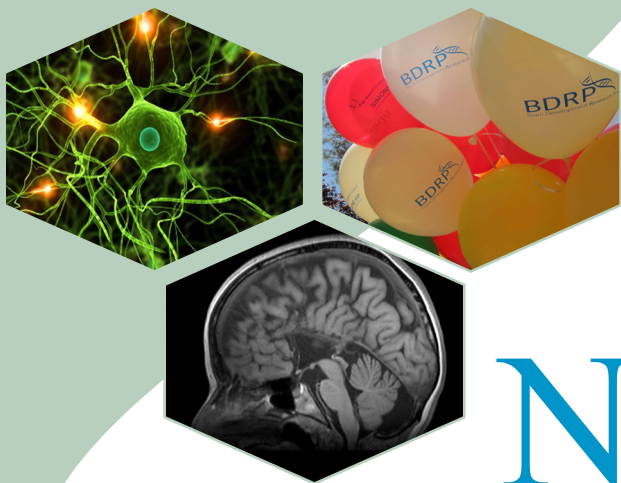




Participating in our research

If you or someone you know is interested in learning more about our research, please call (415) 502-8039 or visit <http://brain.ucsf.edu>. More information can be found on Page 8 at the end of this newsletter.

Newsletter 2012

BRAIN DEVELOPMENT RESEARCH PROGRAM

Dr. Sherr is a board certified pediatric neurologist and directs the Comprehensive Center for Brain Development at the University of California, San Francisco. His research is focused on better understanding the causes of autism, epilepsy, and disorders of brain development.

A warm welcome from Elliott Sherr, M.D., Ph.D.



A warm welcome from the Brain Development Research Program (BDRP) here at UCSF! This is our sixth year providing an annual update, and we're excited to tell you about our research progress and future directions.

Before I go any further, I just want to extend a BIG thank you to all of our study participants and referring healthcare providers. We could not be more grateful for your help by participating in our research programs. Through your substantial support and contributions, we are able to make significant headway towards better understanding neurodevelopmental disorders and devising new treatments to ameliorate the associated difficulties.

In this newsletter, our team will give you an update on

what is going on in the lab and provide you with information about how you can get involved or continue to help! You will hear directly from just a few of the many talented members of our multidisciplinary team who dedicate their time and talents to identifying the genes responsible for brain development and the mutations in these genes that lead to Autism, Epilepsy, and brain development disorders.

In our 2010 newsletter, Dr. Liliana Fernandez had just

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begun using zebrafish to study how genes influence brain development. In her article, Dr. Fernandez discusses her work in characterizing the effects of a novel gene, *TMTC4*, which appears to be associated with AgCC. Dr. Fernandez is working to characterize this gene's function in brain development in zebrafish.

Dr. Jiang Li has been with the BDRP since 2005, and he continues to make remarkable progress in unraveling the genetic causes of brain development through next generation DNA sequencing. In this newsletter, Dr. Li discusses multiple approaches used in our lab to analyze the DNA samples generously donated by our research participants.

Dr. Elysa Marco is a dedicated researcher and clinician who serves as the Director of the Autism and Neurodevelopment Center at UCSF. In addition to providing outstanding clinical care for patients with AgCC, autism, and other neurodevelopment disorders, Dr. Marco regularly contributes to our lab's research efforts. In this newsletter, she'll share with you some interesting findings about cognitive executive functioning in individuals with AgCC compared to individuals without AgCC.

Drs. Dorothy Jones-Davis and Alireza Faridar continue to make advances in autism genetics in animal models. In this newsletter, they describe their research on the ERK signaling pathway, a biochemical pathway thought to be affected in patients with autism spectrum disorder. Their efforts to identify novel biomarkers in autism could lead to earlier diagnosis, novel targeted treatments and improved prognoses.

Julia Owen, PhD has been a tremendous asset to Dr. Pratik Mukherjee's radiology and biomedical imaging lab and to our study in mapping the vast network of connections in the brain. In her article, she highlights some of her exciting new insights in brain networks and pathways in AgCC. Dr.

Owen's "connectome" data is helping us better understand the effects of AgCC on interhemispheric connectivity. Likewise, Leighton Hinkley, PhD continues his groundbreaking work using magnetoencephalography, or MEG, to precisely measure electrical activity in the brain. Through this method of brain imaging, Dr. Hinkley reveals ways in which the absence of the corpus callosum impacts brain dynamics.

In addition to being updated on our existing projects, you will learn about our latest projects and collaborations, such as the Simons Variation in Individuals Project (SVIP) and Epi4K: Gene Discovery in Epileptic Encephalopathies.

Polina Bukshpun and Nick Pojman are the friendly faces of SVIP at UCSF. They have done an incredible job coordinating participants' research visits to UCSF for advanced imaging. In this newsletter, Polina and Nick describe the methods and goals of the study. We've only just begun, and we're already excited about some

of our preliminary findings! Sahar Esmaeeli-Nieh, PhD and Maura Madou, MD will tell you a little bit about our lab's work with Epi4K. Epi4K was made possible by the Epilepsy Phenome/Genome Project (EPGP), the largest study ever conducted on the genetic causes of epilepsy. Sahar and Maura will aid in clinically and genetically analyzing EPGP's Infantile Spasms cases.

This is just a sampling of the many efforts underway in the lab, and there are many other lab members whose work is essential to making these discoveries: Gilberto da Gente, Corby Dale, Eric Rider, Mari Wakahiro, Tracy Luks, Anne Findlay, Rita Jeremy, Brianna Paul, and our newest research coordinator, Katie McGovern, who helped compile these articles and put this newsletter together. I would also like to especially thank Dick Howell, a longtime volunteer and an inte-

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From left to right: Maxwell Cheong, Pratik Mukherjee, Hana Lee, Rita Jeremy, Mari Wakahiro, Elliott Sherr, Julia Owen, Polina Bukshpun, Brianna Paul, Tracy Luks, Nick Pojman, and Katie McGovern

gral member of our team. Dick has dedicated a substantial amount of his time, energy, and resources over the past six years to help implement systems to digitize, organize and archive our extensive collection of brain imaging data. We are forever grateful for his willingness to roll up his sleeves and share his expertise.

We hope you enjoy reading this issue and find it helpful for understanding the BDRP's day-to-day efforts towards better understanding brain development and associated disorders. Please feel free to contact us if you have any questions or if you're interested in becoming involved. As I mentioned before, our work would not be possible without those who participate in and support our research. Therefore, if you or someone you know has been diagnosed with or is thought to have Autism, Epilepsy, or other brain development disorder, please call us at 415-502-8039 or visit

our website at <http://brain.ucsf.edu>.

Finally, let me say that it has been a real privilege to work with all of our research participants over these years. I am humbled by the amount of time and energy that you all have selflessly devoted towards advancing our research. Your contributions do not go unnoticed. Thank you for rallying around us and for your continued support.



Elliott Sherr, M.D., Ph.D.
Principal Investigator

TMTC4: Candidate Gene for the Development of the Corpus Callosum

By Liliana Fernandez, M.D.

The genetics of the agenesis of the corpus callosum (ACC) is variable and reflects the underlying complexity of the development of this bundle of nerve fibers in the brain. In previous studies, we have identified a novel gene, TMTC4, in patients with ACC. TMTC4 is highly expressed in the developing brain and specifically in midline brain structures responsible for development of the corpus callosum. We are using the zebrafish as a model system to characterize this gene and understand its function in the development of the corpus callosum. The zebrafish is an attractive model for developmental biology and genetics because it is a vertebrate with conserved organization of common organs and tissues similar to humans, including the brain and the spinal cord. Moreover, zebrafish produce large clutches of externally fertilized and transparent eggs that develop rapidly and synchronously,

with the first day of zebrafish development being approximately equivalent to the first trimester of mammalian development.

By injecting molecules, called morpholinos, that block TMTC4 expression, we have shown that after 48 hours, by blocking the TMTC4 gene the embryos show a very small head and small or no eyes. By co-injecting the morpholino that blocks TMTC4 expression and TMTC4, we are able to reverse these abnormalities seen in the embryos. These findings indicate the importance of this gene. Examples of these findings are shown in Figure 1. By this method, we will also determine if the changes identified in TMTC4 in the DNA of our patients with ACC are pathogenic.

To further investigate this gene, we are planning to

perform more studies using the zebrafish and human cell lines to understand the role of this candidate gene in the development of the corpus callosum. Consequently, this will lead to understanding the pathways involved in the development of this fiber bundle and the pathogenesis of ACC and related disorders.

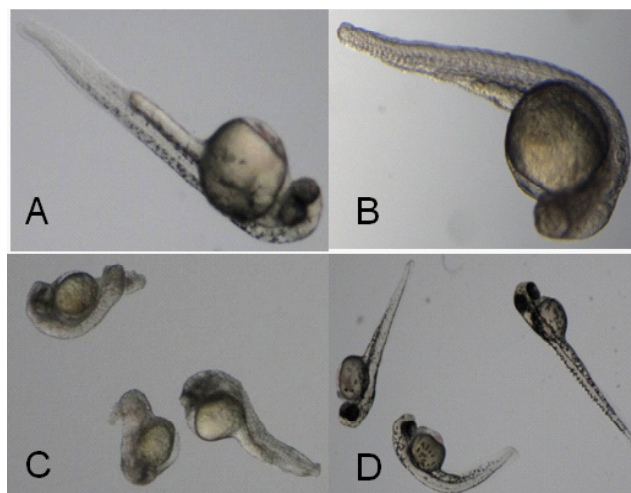
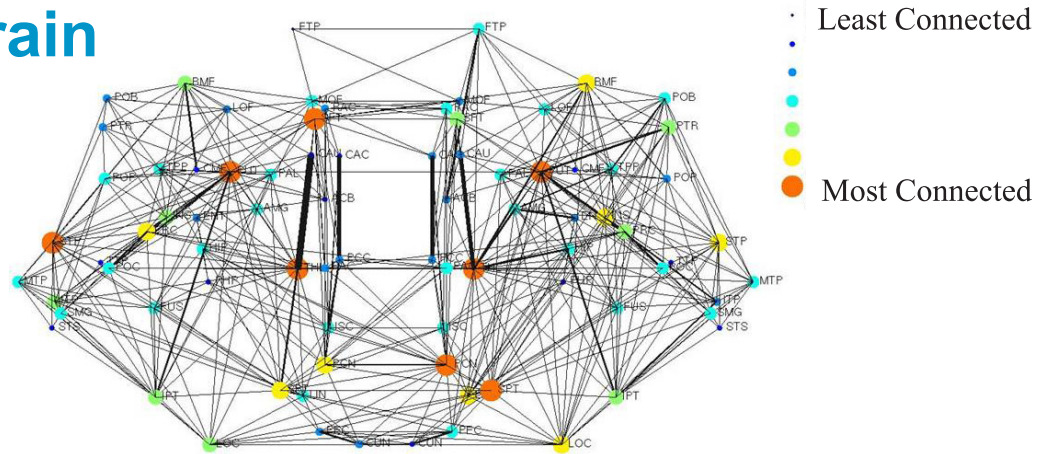


Figure 1. TMTC4 Expression in Zebrafish
A. Non-injected embryo
B. Embryo injected with specific control
C. Embryo injected with morpholino that blocks TMTC4
D. Embryo co-injected with morpholino that blocks TMTC4 + TMTC4

Mapping the Brain

By Julia Owen, PhD

The brain can be thought of as a highly connected network, where white matter tracts are the “wires” by which functionally specialized areas of the brain can send and receive information to/from other regions. Diffusion-weighted MRI (also called diffusion tensor imaging; DTI) enables the imaging of the white matter tracts in the human brain, from which, we can construct the network of white matter connections between various regions of the brain for a particular individual. This global network is often referred to as the “connectome”. The goal of this project is to understand how the absence of the corpus callosum, as seen in subjects with AgCC, affects the network architecture of the brain. Clearly, lacking the major white matter connection between the two hemispheres of the brain will affect cross-hemisphere communication and connectivity, but we are also interested to learn how the individual hemispheres might be rewired due to the absence of the corpus callosum. Borrowing mathematics from the



This is an example of the network of brain regions seen in a healthy control. Each circle represents a brain region responsible for a particular function. The size and color of the circles denotes how connected each region is with all the other regions. The lines between the regions denote the presences of white matter connection.

field of network analysis (a growing field in this age of social networking and the internet), we can approach this biological system with quantitative tools to assess which areas of the brain are under-connected, over-connected or equally connected in the AgCC population as compared to control subjects.

We are finding some interesting differences, most notably that as expected the cross-hemisphere connectivity is drastically affected in AgCC, the within hemisphere connectivity is affected in a much more subtle way. Also, the degree to which the within hemisphere connectivity is altered in AgCC is highly variable between subjects. This means that

there are multiple, variable compensatory “rewiring” strategies in individuals born without a corpus callosum. In the future, we will look for correlations between the variation of the brain network architecture and the variation of cognitive performance in AgCC. This will help us better understand the link between the architecture of the brain and behavior. In the long term, connectome data could eventually help inform clinicians’ treatment methods with AgCC.



Dr. Owen is a post-doctoral fellow working with Dr. Pratik Mukherjee in the Radiology and Biomedical Imaging Department at UCSF.



Magnetoencephalography (MEG) is an imaging technique used to measure the magnetic fields produced by electrical activity in the brain.

Measuring Brain Function in AgCC using MEG

By Leighton Hinkley, Ph.D.

The human brain is a complex organ that operates on many different levels. It produces thoughts, stores memories, regulates emotions, and participates in a myriad of other processes. In our lab, we use a novel brain imaging technique, magnetoencephalography (MEG), to better understand how congenital loss of the corpus callosum (AgCC) impacts brain dynamics.

Two ongoing projects in the lab look separately at 1) how brain regions cooperate with each other in AgCC, and 2) how activation in

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the brain is related to language processing in AgCC. Using MEG, we were able to identify that individuals with AgCC show reduced functional coupling between brain regions, and the degree of coupling is related to deficits in processing speed and problem solving. Furthermore, results from this study elucidate that, in AgCC, cooperation between brain regions within a single hemisphere is reduced to the same degree of cooperation between brain regions across the two cerebral hemispheres. Thus rewiring in AgCC has broad-ranging effects on how different regions of the brain communicate with each other (see section: Mapping Neural Connectivity in AgCC). In the MEG study of

language processing, we observed that individuals with AgCC recruit their right hemisphere for speech reception and production, in comparison to patterns of left-hemisphere language dominance observed in typically developing individuals. Collectively, these findings allow us to use MEG to relate brain dynamics to cognitive impairments observed in AgCC, expanding the opportunity for the development of diagnostics and treatment of the condition.

Dr. Hinkley is a post-doctoral fellow working with Dr. Sri Nagarajan in the Radiology and Biomedical Imaging Department at UCSF.



Unraveling the Code: Approaches to DNA Sequencing

By Jiang Li, M.D., Ph.D.

In order to identify the genetic causes of brain development disorders, our lab uses next generation DNA sequencing to search for candidate genes. Candidate genes are those that are suspected to be the cause of disease. Recent large scale sequencing efforts using DNA from patients with complex diseases, such as Agenesis of the Corpus Callosum and Aicardi Syndrome, compared to DNA from controls suggest that rare mutations play a major role in abnormal brain development.

Our lab utilizes varied approaches and analytical techniques to identify these rare mutations. One approach is to sequence, filter and compare DNA from multiple unrelated, affected individuals. Through this technique, we are able to identify rare single-nucleotide polymorphisms, or SNPs. These are single alterations in DNA sequence.

While SNPs occur often and contribute to genetic variability in the human population, they can also lead to the development of disease. Therefore, we sequenced DNA samples from eighteen participants with Aicardi Syndrome, filtered out common SNPs observed in the population, and successfully identified hundreds of rare SNPs to examine more closely.

A second approach is to sequence DNA from an affected individual and both biological parents, otherwise known as trios. This approach can be used to pinpoint de novo mutations, or new mutations that are not present in either parent. Additionally, we aim to test the hypothesis that Aicardi Syndrome arises from somatic mosaicism. Somatic mosaicism is when different tissue types are genetically distinct, and may be caused by DNA mutations, chromosomal abnormalities, and other genetic alterations. The potential consequences are varied and often deleterious. To test whether or not somatic mosaicism is involved in Aicardi Syndrome, we are expanding the trio approach to a tetrad approach. This means, we are collecting two different tissue samples from the patient with Aicardi Syndrome, and one tissue sample from each biological parent. One last promising approach is whole exome sequencing which enables us to selectively sequence important regions of DNA that code for protein synthesis. Variation in this coding region may have functional consequences that lead to disease. We have sequenced whole exomes in patients from two consanguineous families with autosomal recessive callosal agenesis. Several candidate genes have been identified, and we are now sequencing these candidate genes in other patients with similar clinical characteristics.

The Sherr Lab continues to use the latest technology and DNA sequencing techniques to bring to light the genetic causes of brain development disorders. Through our exhaustive and multifaceted effort, our intent is to better understand the genes responsible for brain development and identify the alterations in DNA sequence that lead to callosal agenesis/dysgenesis, Aicardi Syndrome, and other disorders of brain development.

How Does AgCC Impact Executive Function?

By Elysa J. Marco, MD

Individuals with agenesis of the corpus callosum (AgCC) frequently experience challenges in cognitive skills that are generally termed “executive functions.” Executive function refers to higher order thinking abilities such as the ability to plan, solve problems, switch flexibly between two tasks, and inhibiting impulses that arise. In collaboration with our colleagues at the California Institute of Technology and Fuller Theological Seminary, we have begun to answer the questions of WHY individuals with AgCC have executive function challenges and HOW we can start helping.

We recently focused on two particular executive function abilities: 1) the ability to inhibit a pre-potent response and 2) cognitive flexibility. To be specific, when looking at a printed word, most individuals will automatically read the word. So if you were asked to read the word “red” aloud, you would say “red.” However, if you were asked to name the ink color of this word: **green**, your first impulse would be to say “green” because you are reading the word as opposed to naming the color (red) as instructed.

RED

GREEN

BLUE

This has been verified in hundreds of experiments, and it is actually quite challenging to resist the impulse to name the color! Our second executive function of interest is cognitive flexibility, or the ability to rapidly shift between two sets of instructions. We used two cognitive flexibility tasks, one that involved shifting between reading a printed color word and nam-

ing the color of the ink, and the second involved connecting alternating numbers and letters that were randomly spread over a page. The participant draws a line from 1 to A to 2 to B and so on, alternating between sequencing numbers and sequencing letters.

In this study, 36 individuals with AgCC who have IQ’s within a typical range were compared to 56 age, IQ and gender matched healthy individuals to investigate their cognitive flexibility and inhibition ability. What we found was rather surprising. While the AgCC group, which included both individuals with complete and partial AgCC, generally scored lower on these executive function tasks than the controls, their challenge was not the inhibition or flexibility but rather the overall speed of processing. When the ability to name colors in general was taken into consideration (the AgCC group was slower than the control group), the AgCC and control group no longer appeared different in their ability to inhibit the pre-potent impulse to read a word while naming a color. Similarly, when the baseline ability to read words and name colors was factored in, the AgCC group was no longer impaired in their ability to switch flexibly between instructions. The number and letter-sequencing task confirmed this observation that the difference between groups primarily lies in their ability to sequence number and sequence letters rather than the ability to shift between the tasks. So in answer to the question of WHY individuals with AgCC have challenges on task of cognitive flexibility and inhibition, one answer is that their overall processing of information is slower, on average, than controls.

This observation of slow processing speed is not surprising given the



Elysa Marco, MD

Dr. Marco is a child neurologist and Director of Research for the Autism and Neurodevelopment Center at the University of California, San Francisco. Her research focuses on autism, sensory processing disorders, and agenesis of the corpus callosum.

difference in brain region connections that have been described by our group and others. However, it is particularly important to realize the consequence of this slowing on higher order abilities for considering treatment interventions. The simplest intervention, allowing children to have additional time to understand presented material and produce responses, is an adaptation that can easily be implemented and will likely benefit learning, group engagement, and frustration related behavior. We are also beginning to explore computer brain training programs that have been pioneered in other patient groups with processing speed deficits. This project represents our ongoing collaborative efforts to understand the cognitive and behavioral challenges of individuals with AgCC and to move toward creative solutions.

To learn more about the Autism and Neurodevelopment Program, please visit their website at <http://anp.ucsf.edu>

Disrupted Biochemical Signaling in Autism Spectrum Disorder

By Dorothy Jones-Davis, PhD & Alireza Faridar, MD



Autism is a neurodevelopmental disorder of unknown etiology. Affected individuals may have language disruption, impaired social functioning, and self-stimulatory and/or repetitive behaviors. An estimated one out of every 150 children has some degree of autism, and as many as 1.5 million people in the United States currently live with the disorder.

Advances in autism genetics and in the study of animal models provide evidence to suggest that some biochemical pathways are commonly affected in autistic patients. The ERK signaling pathway is one such candidate that has recently received increased attention. This pathway mediates the transmission of signals from the cell surface to the cytoplasm and nucleus of the cell. Activity levels of the ERK signaling pathway can regulate diverse cellular process such as proliferation, differentiation and cell survival. In the nervous system, ERK signaling appears to be involved in a diverse array of activity-dependent neuronal events including cognitive function and memory formation. Therefore, we have hypothesized that ERK signaling pathway is disrupted in our patients who fall on the autism spectrum.

To evaluate this hypothesis, we first compared the activity level of this pathway in an animal model of autistic-like behaviors, and compared these mice to “control” mice that did not exhibit these behaviors. As we predicted, the activity level of the ERK signaling pathway was significantly increased in the brains of the mice exhibiting autistic-like behaviors, but not in the control mice. Finally, by testing the brain of intercrossed mice (autistic mice×control mice), we have shown that there is a correlation between mice who exhibit more autistic-like behaviors and the degree of ERK signaling pathway activity.

We are very excited about these results, as they may not only be helpful in the development of a novel diagnostic marker (biomarker) for autism, but may also be helpful in determining novel therapeutic targets in autism. Our future goal is to evaluate whether the ERK signaling pathway can be used as a biomarker to aid in the diagnosis of autism, and possibly for monitoring treatment efficacy.

Gene Discovery in Infantile Spasms

By Sahar Esmaceli-Nieh, Ph.D. & Maura Madou, M.D.

Infantile spasms is an epilepsy syndrome that typically presents in young infants. The diagnosis is made when there are characteristic brief jerking movements that often occur in clusters and a typical erratic EEG pattern referred to as hypsarrhythmia. Patients are then grouped into symptomatic or cryptogenic infantile spasms subcategories. Symptomatic spasms are associated with an underlying cause such as structural brain abnormalities, perinatal asphyxia and/or a number of genetic syndromes including tuberous sclerosis (most common), neurofibromatosis and Down syndrome. When no underlying cause can be found, the patients receive a diagnosis of cryptogenic infantile spasms—a diagnosis that is given to roughly one third of all cases. As part of the Epilepsy Phenome/Genome

and Epi4K projects, we aim to further categorize patients with cryptogenic infantile spasms to develop a better understanding of the genetic factors involved, enabling researchers to translate genomics into biological diagnostics, individualized treatments, and target identification for development of new therapeutics.

It is well known that many forms of epilepsy are strongly influenced by genetics; however, there has been relatively little progress in identifying the genetic differences that contribute to most forms of epilepsy, including cryptogenic infantile spasms. For this purpose, the Epilepsy Phenome/Genome Project has assembled the largest cohort of patients with cryptogenic infantile spasms worldwide to date. From a clinical (or phenotypic) standpoint, we will be ana-

lyzing medical records extensively to identify patterns within patient groups that might serve as clues to genetic changes that correlate with these findings. As part of Epi4K, we will use modern genetic technologies to systematically screen the patients' genomes to search for de novo or rare pathogenic mutations that influence risk of cryptogenic infantile spasms. The discovery of new causes for cryptogenic infantile spasms will allow us to develop a better understanding of how these genes function in brain development and lead to more accurate patient grouping for interdisciplinary clinical intervention. Through this research, we hope to develop novel therapeutics that will lead to the best possible seizure control and developmental outcomes for patients.

Genetics First Approach to Autism: Advanced Brain Imaging and 16p11.2

By Polina Bukshpun & Nick Pojman

The 16p11.2 copy number variations (CNV) are two of the most common genetic events associated with autistic spectrum disorders (ASD). We, along with a network of scientists from 4 other research hospitals have just launched a new study aimed to better understand the medical, cognitive, and behavioral features of individuals with 16p11.2 deletions or duplications. We hope, by studying a group of individuals who all share the same genetic change, to discover novel insights that explain how disrupted brain function can lead to the behavioral and cognitive changes of ASD.

Dr. Sherr and the UCSF team serve dual roles, directing brain imaging at all five hospitals and also serving as one of two hospitals that will ask participants to undergo a comprehensive and detailed set of brain imaging tests. These tests will include diffusion tensor imaging (DTI; a means to “see” the physical connections in the brain), functional Magnetic Resonance Imaging and magnetoencephalography (fMRI and MEG; two methods used to observe how the brain works when asked to perform basic tasks like hearing spoken

language or seeing pictures). These imaging assessments will also be paired with a comprehensive neuropsychological battery, helping us assess cognitive domains commonly impaired in autism and known to be affected in 16p11.2 patients, including language processing, executive function (how we organize our thoughts), face recognition (how our brains respond to faces is thought to be central to who we are as social beings and something that is impaired in ASD) and motor processing.

We hope that this will provide a better understanding of the brain structure-function relationship in this cohort of individuals and may have implications for diagnosis and treatment of the more general cohort within ASD. A more detailed description of the study was just published and is available free online: [http://www.cell.com/neuron/abstract/S0896-6273\(12\)00175-4](http://www.cell.com/neuron/abstract/S0896-6273(12)00175-4). We can also send you a PDF by email if you have difficulty accessing this site.

SIMONS VIP

SIMONS VARIATION IN INDIVIDUALS PROJECT



Our team at UCSF is presently enrolling healthy volunteers ages 7 and older to participate. The goal of this study is to better understand and improve clinical care for individuals with autism and related developmental disorders. For more information, please contact us:

(415) 502-0183

simonsvip@neurology.ucsf.edu



www.facebook.com/UCSFSVIP



Participate in our Research

If you or someone you know have been diagnosed with autism, epilepsy, or a disorder of brain development, please follow these steps to learn about how to become involved and participate in our research.

Step #1 Contact one of our research coordinators by calling **(415) 502-8039** or visiting <http://brain.ucsf.edu/contact-us>.

Step #2 Our research team will determine eligibility through the following ways:

- Taking a look at your/your child's most recent MRI or CT scan of the brain. You can either mail a copy to us or complete a release of records form, and we'll request it directly from the medical institution where it was performed.
- Asking you to complete an intake survey or initial screening questionnaire.

Step #3 If eligible, you will receive more information about how to enroll and complete all of the required research activities.

Step #4 Keep in touch! Let us know how you or your family is doing from time to time. We'll also keep you posted on our findings.