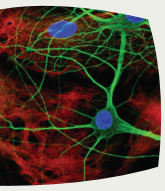

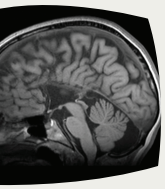

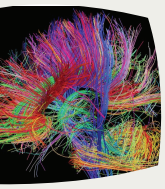

Newsletter

BRAIN DEVELOPMENT RESEARCH PROGRAM **2016**

Dr. Sherr is a board certified pediatric neurologist at the University of California, San Francisco. His research is focused on better understanding the causes of autism spectrum disorder, epilepsy and disorders of brain development.

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

Hello again and welcome from the Brain Development Research Program (BDRP) here at UCSF! Before I go any further, I would like to extend a big thank you to all of our study participants and referring healthcare providers. We are incredibly grateful for your help and participation in our research programs. We are able to make significant progress towards better understanding neurodevelopmental disorders through your contributions and substantial support.

It is hard to believe that it has been almost four years since we last updated you on all of the projects we have been working on. During this time the lab has been quite productive: publishing or co-publishing over 40 peer reviewed articles on many topics important to our field including the genetics and basic biology of brain malformations (agenesis of the corpus callosum), epilepsy, and autism spectrum disorder, as well as work on brain imaging and clinical phenotyping of individuals with autism spectrum disorder including a specific subgroup of individuals who all carry

▶ Continued on next page

Participating in our research

Interested in one of our studies or know someone who might be? We would love to have you participate! Contact Brieana Fregeau (Brieana.Fregeau@ucsf.edu) or Talia Berson (Talia.Berson@ucsf.edu) or give us a call at (415) 502-8039 and we would be happy to provide you with more information! For more information, visit our website at http://brain.ucsf.edu.

Find us on Facebook
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a 600 kilobase deletion or duplication at chromosome locus 16p11.2. While our research provides some answers, there are many new questions raised from our work, that may point us not only toward a better understanding of these neurodevelopmental disorders, but also provide insights into developing novel therapeutics. Below, each of the members of our lab has provided a short summary of their ongoing work. I hope you find these projects as exciting and interesting as we do. Do not hesitate to reach out to us if you have any questions or would like copies of the published manuscripts that have come out of the lab. We have listed a select number of these on the last page. Thank you for all your help and we look forward to hearing from you in the future.

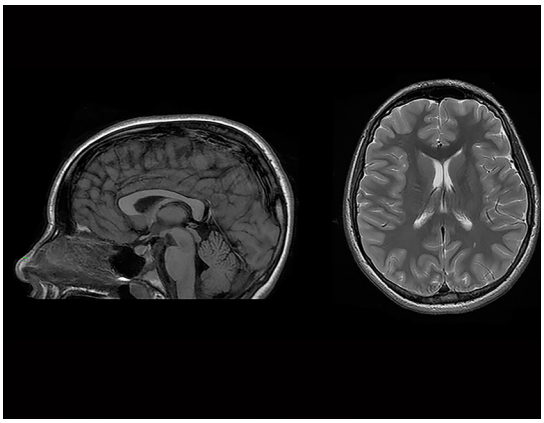
Cordially,

Elliott Sherr, M.D., Ph.D.

Brain Imaging and Cell Signaling: Insights into the Biology of Autism Spectrum Disorder

By Talia Berson

We are looking forward to beginning our newest project, "Brain Imaging and Cell Signaling: Insights into the Biology of Autism," which will examine the link between macrocephaly and autism spectrum disorder (ASD). It has long been acknowledged that ASD and macrocephaly, (head size in the 97.5th percentile or above), can be correlated in a significant subset of children with ASD. To further understand this connection, we will be recruiting participants with a range of head sizes 6 years and older, both those with ASD and neurotypical controls. We will perform cognitive testing, and enlist volunteers to undergo brain scanning with magnetic resonance imaging (MRI) and magnetoencephalography (MEG). We are excited to begin this process and would be happy to speak with you about any questions you may have.



Blood-based Biomarkers for Autism Spectrum Disorder

By Emanuela Arilli, Ph.D., and Sigrid Elfstroem

Autism spectrum disorder (ASD) refers to a group of complex and heterogeneous disorders of brain development. It is characterized by persistent deficits in social communication and social interaction and restricted, repetitive patterns of behaviors. It is estimated to affect one in 68 children. Therefore, a major scientific challenge is to discover a biological marker that could allow for an earlier and more effective diagnosis, and with that a range of early interventions. Advances in ASD genetics and in the study of animal models provide evidence to suggest that some biochemical pathways are commonly affected in patients with ASD. Our early results demonstrate that the activity level of specific selected intracellular signaling pathways is significantly altered in the peripheral blood of syndromic ASD patients. In many cases, individuals with ASD have elevated activity, though others have significantly decreased activity. Work is ongoing to elucidate whether the same is true for much younger children with ASD, with the ultimate goal of showing that these blood measurements (often called biomarkers) can be predictive of ASD status in presymptomatic toddlers. Please contact Talia Berson for more information regarding study design and who is eligible to participate.

C12ORF57 and Agenesis of the Corpus Callosum

By Ruiji Jiang

The genetics behind agenesis of the corpus callosum (ACC) are varied, which reflects the complexity of development of this large tract of nerve fibers in the brain. The Sherr lab, in collaboration with several other groups recently discovered a new gene, C12ORF57, in a cohort of patients who present with partial or complete agenesis of the corpus callosum. These patients also have optic nerve coloboma (a failure of formation of the optic nerve), intellectual disability, and seizures along with some patients also having bone deformities and diabetes. While C12ORF57 is highly conserved through several species, it has no known function, nor does the sequence of the amino acids in the protein resemble that seen in other known proteins with known functions.This makes C12ORF57 a particularly interesting and mysterious gene to study since we do not know its function or how it plays a role in the formation of the corpus callosum.

To further that goal, we are doing studies such as "Yeast-Two-Hybrid," which have already given us promising results pointing us towards other proteins interacting with C12ORF57. This technique can give us key insights into the protein's function. We also are working to characterize and develop a mouse model where the gene has been engineered to be "knocked out," or rendered non-functional. We hope that this animal model will allow us to understand how changes in development of the corpus callosum and of the brain of these animals occurs and how this impacts patients with this condition. Moreover, we hope that these discoveries will have broader implications for brain development and function in children with ACC and autism spectrum disorder.

De novo mutations of RERE cause a new genetic syndrome whose features overlap those associate with 1p36 deletions

By Brieana Fregeau

Deletions of chromosome 1p36 affect one in 5,000 newborns and are frequently identified because of intellectual disability associated with defects involving the brain, eye, ear, heart and kidney. Within the 1p36 region there is a gene called RERE, a widely expressed gene that has been shown to positively regulate retinoic acid signaling. Animal models suggest that RERE deficiency may contribute to many of the structural birth defects and medical problems seen in individuals with 1p36 deletion syndrome, but human evidence supporting this role has been lacking.

Together with a team of collaborators, our lab recently described 10 individuals with intellectual disability, developmental delay and/or autism spectrum disorder who carry rare, putatively damaging, changes in RERE. Associated features that were seen often in these individuals included weakness, seizures, behavioral problems such as autism, structural brain anomalies (such as a thin corpus callosum), eye defects, and congenital heart defects. These changes in a baby's development are also seen in individuals with deletion of the chromosomal region, 1p36, which includes the gene RERE. RERE-deficient mice also have the same structural brain and eye anomalies. Taken together, our findings suggest that mutations in RERE cause a novel genetic syndrome that includes, but is much broader than, ACC. This work is now accepted for publication in the American Journal of Human Genetics.

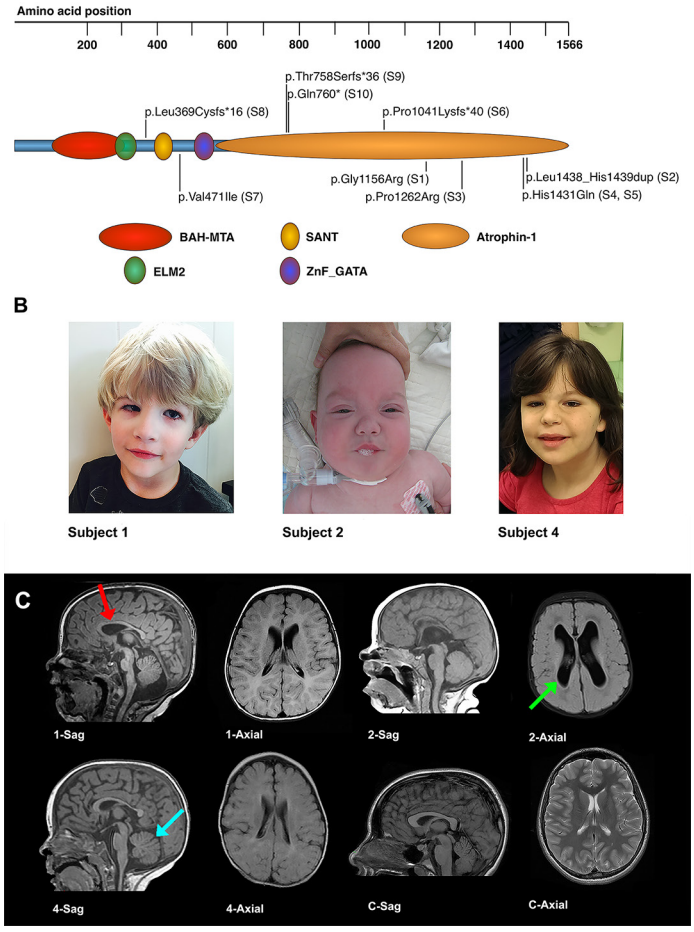
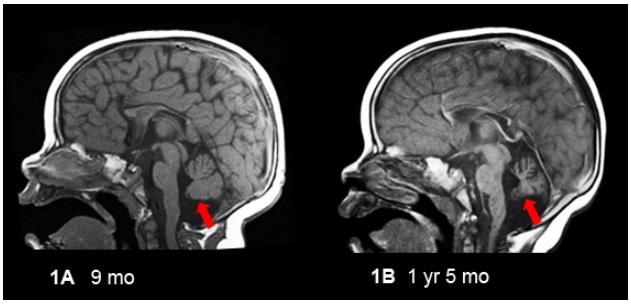


Figure 1. Molecular changes and selected clinical findings of individuals with putatively deleterious changes in RERE. A) The predicted locations of domains within the RERE protein are presented along with the locations of the RERE protein changes seen in Subjects 1-11. B) Craniofacial changes noted in subjects. C) Sagital (Sag) and axial brain MRI scans of Subjects 1, 2, 4, and a control demonstrating characteristic findings.

De novo mutations in KIF1A cause progressive encephalopathy and brain atropy

By Sahar Esmaeeli, Ph.D.



The causes of developmental encephalopathies are complex and remain poorly understood. Arriving at a diagnosis can have important implications for clinical management and prognosis. First-line genetic testing includes high-resolution chromosomal analysis, genomic microarray, directed single gene, and gene panel testing along with biochemical assays for inborn errors of metabolism. For patients who remain undiagnosed after these tests, whole exome sequencing (WES) is now a clinically available and indicated option.

To determine the cause and course of a novel syndrome with progressive encephalopathy and brain atrophy in children, clinical whole exome sequencing was performed. Six patients were identified with de novo missense mutations in the kinesin gene KIF1A. This is a motor protein that moves cellular "cargo" from the cell body to the end of the axon terminal in neurons in the brain. These mutations were evaluated by computational approaches that predicted that the "engine" of this motor (ATP hydrolysis) was likely to be prevented from working by these mutations (dominant interfering mutations). These mutations were then tested by an in vitro microtubule-gliding assay, showing that these dominant mutants were non-motile in this gliding assay.

All six subjects had severe developmental delay, hypotonia and varying degrees of hyperreflexia and spastic paraparesis. Microcephaly, cortical visual impairment, optic neuropathy, peripheral neuropathy, ataxia, epilepsy, and movement disorders were also observed. All six patients had a degenerative neurologic course. Progressive cerebral and cerebellar atrophy were seen on sequential MRI scans, findings that were all concordant with the genetic etiology.

In summary, we demonstrated that de novo mutations in KIF1A cause a degenerative neurologic syndrome with brain atrophy. Computational and in vitro assays differentiate the severity of dominant de novo heterozygous versus inherited recessive KIF1A mutations. The de novo mutations have a profound effect on axonal transport; the likely cause of observed progressive neurologic impairment in patients. This work was recently published in the Annals of Clinical and Translational Neurology.

The Role of ANK2 in Autism

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

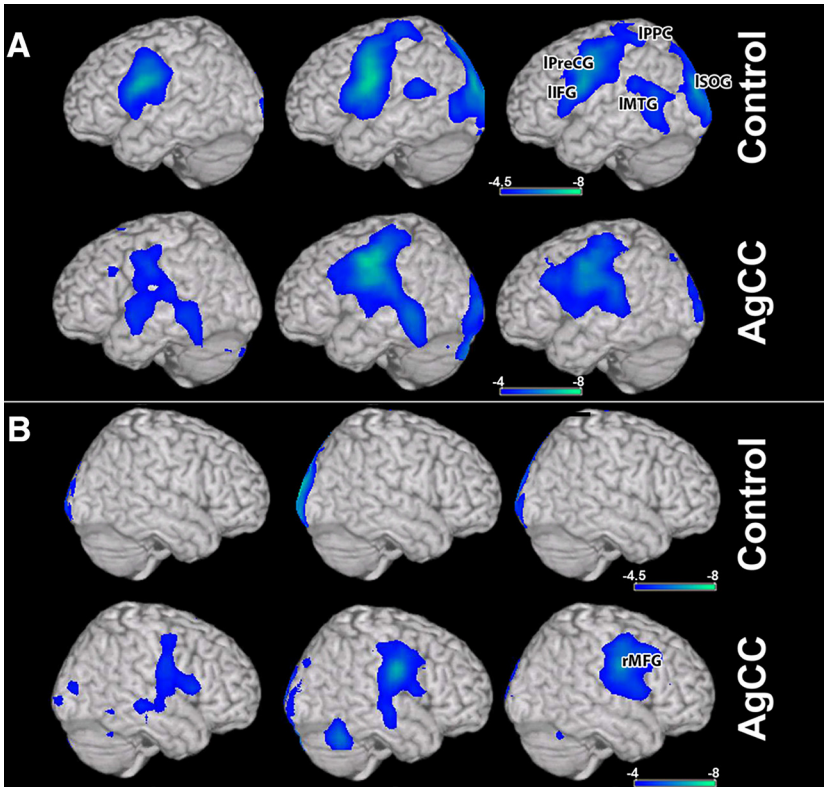
ANK2 encodes a member of the ankyrin family of proteins (Ankyrin B) that link integral membrane proteins (e.g. L1CAM) to the underlying spectrin-actin cytoskeleton. Ankyrins play key roles in activities such as cell motility, activation, proliferation, cell-cell contact, and the maintenance of specialized plasma membrane domains. Mutations in ANK2 have been found to cause autosomal dominant long QT syndrome 4 and cardiac arrhythmia syndrome in humans. Recent next generation DNA sequencing projects in ASD cohorts (the Simons Simplex Collection) have revealed several heterozygous de novo ANK2 missense and nonsense mutations marking ANK2 as a 'high-confidence' ASD candidate gene. Additionally, our lab has identified multiple individuals who not only have cognitive and behavioral deficits, but are also missing the corpus callosum (agenesis of the corpus callosum; ACC), a structure comprised of nearly 200 million axons connecting the two cerebral hemispheres. These genetic findings highlight the importance of ANK2 in neurodevelopment, but how a mutation of ANK2 leads to these brain disorders is still unknown. As a first step to address this question, we reported an expected and significant decrease in expression of ANK2 mRNA and protein in patients compared to control PBMC's. We also investigated the abundance of the neuronal adhesion protein, L1CAM. This protein has been shown to bind to ankyrin B, and the protein half-life was dependent on ankyrin B "anchoring" L1cam to the plasma membrane. Thus, we found that L1CAM protein abundance is decreased in patients with ANK2 mutations [one with a balanced

chromosomal translocation through ANK2 t (4;8)(q25; q23), and in two other Autism patients with nonsense mutations in ANK2] and that L1CAM expression is rescued after transient transfection of ANK2 into a cell line from one patient carrying a nonsense mutation in ANK2. Our results suggest that disruption of the normal interaction and balance between ANK2 and L1CAM may play an important role in autism spectrum disorder and other neurodevelopmental disorders, such as ACC. Moreover, this linkage between ACC and ASD has implications for the mechanistic overlap for these two groups of disorders.

Language Lateralization in Agenesis of the Corpus Callosum

By Leighton Hinkley, Ph.D.

Many functions in the brain are "lateralized," meaning that either the left or the right hemisphere is specialized for a specific task or behavior. The most well known lateralized function is language, where (in over 90% of individuals) the left hemisphere of the brain is designed to process and produce language. How the left hemisphere is chosen during development to specialize for this task is poorly understood, although one potential brain structure that may do so is the corpus callosum, a large band of white matter fibers that bridge the two hemispheres of the brain. If this is true, then individuals with agenesis of the corpus callosum (ACC) who are born with either a partially developed callosum or no callosum at all should not have left lateralized brain function. In a paper recently accepted for publication in The Journal of Neuroscience, we have used an advanced brain imaging technique (magnetoencephalographic imaging, or MEG-I) to examine lateralization for language in individuals with ACC, comparing those against a group of neurotypical individuals with a fully formed corpus callosum. Here, we see that the majority of individuals who were born without a corpus callosum actually use their right hemisphere for language function. Interestingly, individuals with only partial ACC do not specialize a single hemisphere, using both left and right sides of their brains equally. Most importantly, we find that individuals with ACC who use the right side are more likely to have lower verbal intelligence, helping to explain why some individuals with callosal agenesis suffer from language problems. MEG-I is an exciting technique that allows us to look at brain activity as it occurs, and findings that link brain function to clinical presentation provide an opportunity to generate treatments that target physiology to remediate these cognitive impairments.



MEG reconstructions of brain activation in the left hemisphere (top two rows) and right hemisphere (bottom two rows) during a language test in both neurotypical control individuals (Control) and patients with agenesis of the corpus callosum (AgCC). While both the Control and AgCC groups show the same patterns of activity in the left hemisphere, only patients with AgCC show activation in the right hemisphere during the MEG language test.

Brain Imaging in 16p11.2

By Julia Owen, Ph.D.

In a multicenter study funded by the Simons Foundation called the Simons Variation in Individuals Project (VIP), we have been investigating neuronal differences in children and adults with copy number variations at the 16p11.2 gene locus. Both a duplication and a deletion at this location on the genome puts an individual at higher risk for autism, as well as other neurodevelopmental and neuropsychiatric disorders. Here we present our exciting findings using MR imaging.

Changes in the Structure of the White Matter in 16p11.2: Our first focus was on the microstructural properties of the white matter in individuals with a deletion or duplication. Using diffusion MRI, it is possible to detect the diffusion of water on the molecular level in the brain. This provides an indirect measure of the integrity of the axons in the underlying white matter.

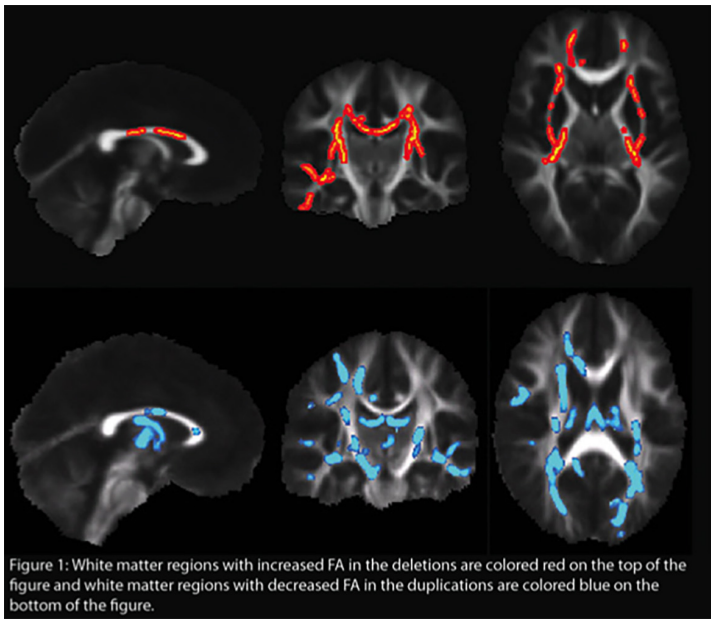


Figure 1: White matter regions with increased FA in the deletions are colored red on the top of the figure and white matter regions with decreased FA in the duplications are colored blue on the bottom of the figure.

The diffusion of the water adjacent to axons that have suffered injury or differ in composition will be altered. The metric fraction anisotropy (FA) provides a quantification of the directionality of the flow of water. FA close to one implies that the water flows only in one direction and FA close to zero implies equal flow in all directions. We found a very striking and intriguing duality in our results. The deletions had increased FA while the duplications had decreased FA in key white matter tracts including the corpus callosum (see Figure 1). These results mimic the head circumference differences between deletions and duplications; deletions tend to have larger heads (with some instances of macrocephaly) while the duplications tend to have smaller heads (with some instances of microcephaly).We do not fully understand the interplay between increased brain size and increased FA in the deletions and how this change could lead to deficits in cognition or behavior. In our future work, we plan to further explore the mechanisms behind this finding. This work is in revision at Human Brain Mapping, due to be published in 2016.

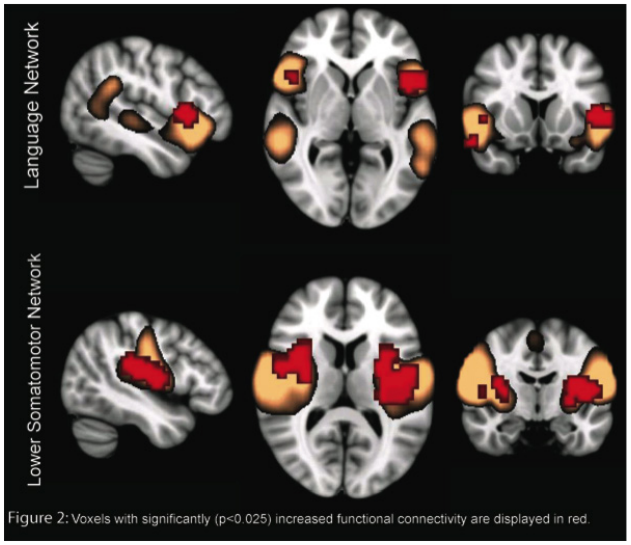


Figure 2: Voxels with significantly (p<0.025) increased functional connectivity are displayed in red.

Increased Functional Connectivity in Language Networks in 16p11.2 Deletions: Using resting-state fMRI, inherent fluctuations in the MR signal can provide a measure of how strongly connected brain areas are to one another. We found increases in connectivity in two networks of brain regions in the deletions: the language network and the speech motor network (see Figure 2). The language network includes Wernicke's and Broca's areas, we found increased connectivity primarily in Broca's area, which is responsible for speech motor planning. The speech motor network consists of motor and somatosensory regions responsible for the control of the lips, tongue, and entire vocal apparatus. These findings demonstrate specificity to the motor planning and execution of speech. Not coincidently, over 50% of the deletions included in this study have a clinically diagnosed articulation disorders and around 40% have a language disorder. We did not find differences in connectivity in these networks in the duplication cohort. In the future, we plan to build on these findings and further investigate the neural underpinnings of speech and language deficiencies in the deletions in order to identify potential therapies for these pervasive deficits. This work is currently being prepared for publication in 2016.

Neuroanatomical Differences Attributed to 16p11.2 Deletions and Duplications: This part of the VIP study included data from 310 participants: 75 deletion carriers, 70 duplication carriers, 56 familial non-carriers (parents and siblings of both deletion and duplication), and 109 healthy population controls. Participants underwent clinically oriented structural MRI scans and completed a battery of cognitive and behavioral tests. MRIs were reviewed by three board-certified neuroradiologists, blinded to group, evaluating the scans for development-related neuroradiological features. We then assessed differences in frequency or prevalence of the various features between the control subjects and the deletion and duplication carriers with statistics. In comparison to controls (familial non-carriers and population), deletion carriers were found to have enlarged corpus callosum volumes and a greater likelihood of enlarged cerebellum (called a cerebellar ectopia) and anatomic abnormalities at the juncture of the brain and spine. In assessing impact of these differences on clinical outcomes, we found that deletion carriers with either cerebellar abnormalities had lower scores on a test of communication and those with an increased corpus callosum had worse performance on both measures of social and communication function. In contrast, duplication carriers had a decreased callosal size, decreased white matter volume and their ventricles were larger than usual. Duplication carriers with at least one of the above findings had lower IQ scores. The findings show that clinically evident neuroanatomical alterations distinguish 16p11.2 deletion or duplication carriers from controls and each other, and that these anatomic alterations can predict the degree of impairment within these patient groups. Publication of this work is in progress.

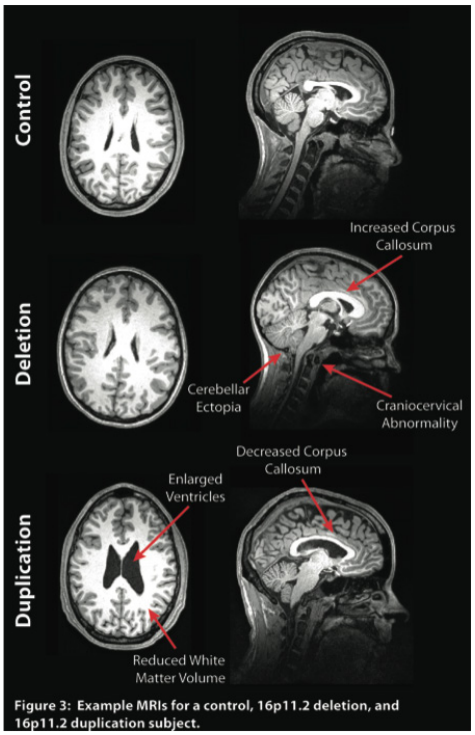
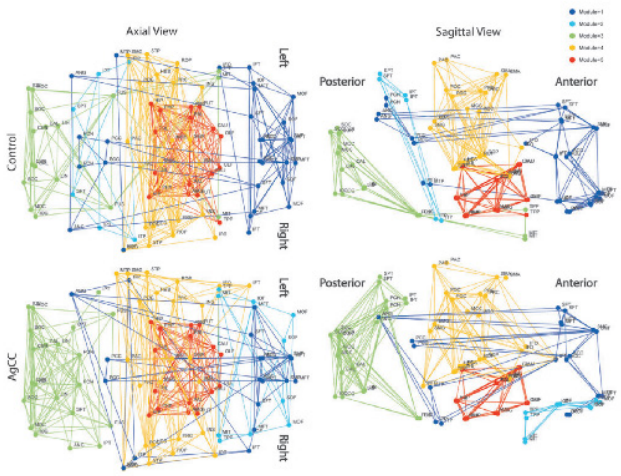


Figure 3: Example MRIs for a control, 16p11.2 deletion, and 16p11.2 duplication subject.

Largely Preserved Functional Connectivity Patterns in Agenesis of the Corpus Callosum

By Julia Owen, Ph.D.



Connectivity diagram for controls (top) and AgCC (bottom). Each circle represents a brain region and each line represent a functional connection between brain regions. The nodes and connections are colored based on the family designation (or modular decomposition). The controls and AgCC groups have strikingly similar families of brain regions demonstrating preserved functional connectivity despite greatly altered structural connections (i.e. lack of or diminished corpus callosum).

In a paper published in Brain Connectivity in 2013 (http://www.ncbi.nlm.nih.gov/pubmed/24063289), we investigated the effect of callosal dysgenesis on functional magnetic resonance imaging (fMRI) resting-state networks. The resting-state networks reflect communication between brain regions essential for the completion of every day tasks. Since alternate white matter connections connect the left and right sides of the brain, we hypothesized that bilateral networks can still be maintained in partial or even complete agenesis of the corpus callosum (AgCC). However, since these alternate routes are likely less efficient than callosal connections, we hypothesized that quantitative measurements of interhemispheric (between the left and right hemispheres of the brain) functional connectivity will be reduced in AgCC compared with matched controls, especially in the most highly interconnected cortical regions that are called the "hubs" of the network. Resting-state networks were extracted from fMRI data of 11 subjects with partial or complete AgCC and 11 matched controls. The results show that the qualitative functional organization of the brain is very similar between controls and AgCC. However, quantitative interhemispheric functional connectivity of hub regions, the precuneus, posterior cingulate cortex, and insular-opercular regions, was significantly reduced in AgCC. We also show that the location of five families of brain regions that are highly connected to one another, known as modules, are largely consistent across the control and AgCC groups. In the figure we show brain regions and the functional connections in a diagram format, where each circle is a brain region, a line represents a functional connection, and the colors denote one of five families of brain regions. Hence, the reduction or even complete absence of callosal connectivity does not affect the qualitative organization of bilateral brain networks, although quantitatively reduced functional connectivity can be demonstrated by measurements within bilateral cortical hubs, supporting the hypothesis that indirect less efficient pathways are utilized to preserve coordination and communication between the left and right hemispheres. Future work will explore any subtle variations in connectivity from individual to individual that could explain differences in cognitive and social abilities observed in individuals with AgCC.

Whole Exome Sequencing in 100 UCSF Patients

By Maura Madou, M.D.

The diagnostic odyssey for children with neurodevelopmental disorders can be extensive, costly, and exhausting. A careful clinical history and exam by the doctor can narrow the differential diagnosis somewhat, but patients and families are often left without a diagnosis or medical management plan for their loved one(s) even after using all conventional testing such as a brain MRI and chromosomal testing including karyotypes and chromosomal microarrays. Whole exome sequencing, a newer genetic test that became available clinically in 2012, has proven that it is a useful new tool to help solve undiagnosed cases and deliver an answer for families, with a success rate ranging from 20-55%.

Whole exome sequencing allows us to look for mutations in all of the genes that we know code for proteins in our bodies. We recently looked back at the first 103 patients who had whole exome sequencing ordered by our subspecialists at UCSF, and the diagnostic yield of the test was confirmed. Testing led to a diagnosis in our patients 51% of the time (unpublished data). We also looked at the average cost of testing done in a search of a diagnosis prior to exome sequencing, and learned that it was more than $15,000 per patient, while whole exome sequencing can now be done for a little under $6000, suggesting that this approach can provide clinically relevant answers in an economically efficient way. The cost, speed and reliability of whole exome sequencing are rapidly improving; yet due to the continued relatively high cost and variable insurance plan coverage, the test is still not available to a lot of patients searching for an answer. We hope that the work in our lab as well as that by many other groups treating patients with undiagnosed diseases will begin to convince insurance carriers of the diagnostic power of this valuable new clinical tool. Besides the long-term cost effectiveness of the test in the long run for a number of cases; the value of an answer is immeasurable.