SHANK2

Tutorial with Parents (September 26, 2017):
During the summer of 2017 Dr. Marion was contacted by a family he had interacted with 20 years earlier. Their son, JA, was 2 years old at the time, and was showing signs of developmental delay and facial dysmorphia. Attempts at a diagnosis with methodologies available at that time failed. The mother of JA had reconnected with Dr. Marion to tell him that after these many years a diagnosis had been made through newly available genetic tests and JA was reported to have a *de novo* mutation in *SHANK2*. Following this, Dr. Marion contacted Dr. Walkley to ask if there were faculty at Einstein doing research on *SHANK2* mutations or related SHANK proteins as he had learned that these parents were interested to learn more about why a mutation in this gene would cause autism. As a result, Dr. Walkley contacted the IDDRC membership asking for interested scientists to respond. This led to an immediate response from Dr. Bryen Jordan in Neuroscience whose lab was actively working on SHANK proteins and their role in synaptic function. This response by Dr. Jordon and the events that followed marked the birth of the Rose F. Kennedy IDD Gene Team concept.

A Zoom teleconference was held on Tuesday morning, September 26, 2017. Both parents, who live in Miami, joined Bob Marion (clinician), Bryen Jordan (scientist) and Steve Walkley (meeting host) with the meeting taking place in the IDDRC conference room in the Kennedy Center. The Zoom conference began with Dr. Marion reviewing his experience meeting the patient 20 years earlier when the family lived in the Bronx. JA was at the time 2 years old and showing signs of developmental delay and some features of ASD. (See further notes below). No definitive diagnosis was achieved at that time. The family then reviewed their experience with JA over the last 2 decades. This was followed by Dr. Jordan giving a detailed lay description of the gene and its protein and its role in synaptic function, as well as in ways that its loss of function might be alleviated. This review was very well received by the family. Discussions were later initiated on possible funding by the family foundation for research in Dr. Jordan's lab but this did not come to fruition.

Dr. Marion remains in periodic contact with the family.

Patient Description:
JA was initially referred for genetic evaluation at 2 years of age because of developmental delay and dysmorphic facial features. Born via an uncomplicated pregnancy and delivery, his neonatal period was complicated by hyperbilirubinemia which required phototherapy. His development was notable for gross motor delays: he sat in tripod at 9 months, sat unassisted at 1 year and took his first steps at age 2. On physical exam at that time, he was noted to have several dysmorphic facial features (bilateral epicanthal folds, slight upward obliquity of the palpebral fissures, small ears and ear canals, wide, flattened nasal bridge, high arched palate with small, wide-spread teeth), nystagmus and strabismus. His height was at the 5th centile. He had wide feet, curled toes and a pilonidal dimple. Muscle tone was mixed: there was central hypotonia with mild spasticity in the extremities

High resolution chromosome analysis, accompanied by FISH for subtelomeric rearrangement was negative. Because of suspicion of genetic disorder, he continued to be followed. Developmental evaluation revealed global delays, with poor fine motor skills and deficits in gross motor skills. He was noted to walk on his toes and to have uncoordinated gait.

At four years of age, surgery was performed to repair strabismus (he continues to wear glasses). At 14, because of progressive motor and sensory symptoms involving bowel, bladder and lower extremities, tethering of the spinal cord was diagnosed and treated with surgical release.
Repeat developmental evaluations have shown IQ in the mid to high 40s, with islands of higher and lower intelligence. He has an outgoing, friendly personality and a great affinity for music, with ability to play piano and other instruments. However, he has poor daily living skills, requiring assistance in dressing and personal hygiene and poor communication and language skills. He has been diagnosed with ADHD, demonstrates perseverative behavior, and has obsessive/compulsive tendencies. He talks incessantly.

As a young adult, whole exome sequencing revealed two mutations in SHANK2 (c. 3356G>A p.Arg1119Gln and c.2709A>G p.A2709G). One was inherited from his mother. The second arose de novo. It is not possible to tell whether these variants are present in cis or trans.

Disease/Syndrome Features:
In 2010, microarray analysis identified *de novo* copy number variations (CNV) in *SHANK2* in one individual with autism-spectrum disorder (ASD) and one individual with intellectual disability (ID). Specifically, these CNVs were deletions of 69 to 120 kb that resulted in the loss of either both exon 6 and exon 7 or exon 7 alone. Upon further evaluation, the individual with ID was also diagnosed as having ASD. One patient was reported to have a motor delay evident at 5 months and slow reactions and adaptation. The other patient was reported to have bilateral clinodactyly 5^{th} digits and bilateral dysmorphic toes. Subsequently, exons of the neuronal isoform of *SHANK2* were sequenced in a cohort of patients with ID and ASD. This inquiry revealed a further eight variations – one *de novo* nonsense mutation, six inherited missense variants, and a microdeletion [Berkel 2010].

Subjects with *SHANK2* mutations show variable phenotypes. For example, the individuals with CNV deletions both had severe ASD and mild to moderate ID. In two individuals with a P208S substitution, however, one had isolated severe ID and the other had ASD with borderline intelligence. Furthermore, all missense mutations were transmitted by unaffected parents. In two cases, unaffected mothers passed mutations in highly conserved amino acids to multiple male children with ASD, autistic-like traits, or language delay. Despite the absence of ASD, both mothers did show depression and/or anxiety. *SHANK2* mutations also point to the interrelatedness of ASD and ID. 63% of the recruited ASD cases had an IQ below 70, and half of the recruited ID cases were diagnosed as having autistic traits during follow-up [Berkel 2010]. In another study of patients with *SHANK2* mutations and ASD, researchers found that patients with *de novo* mutations in *SHANK2* carried additional inherited CNVs in chromosomal regions and specific genes

previously associated with neuropsychiatric disorders. They therefore argue that *SHANK2* mutations underscore the importance of modifier genes and support a “multiple hit model” for ASD [Leblond 2012].

Protein/Pathway:
SH3 and multiple ankyrin repeat domains protein 2, *SHANK2*, encodes a synaptic scaffolding protein that localizes to the postsynaptic density (PSD) of excitatory synapses in the central nervous system. SHANK2 belongs to a family of such proteins that includes SHANK1 and SHANK3, and *SHANK3* has also been associated with ASD. SHANKs and HOMER form a mesh-like matrix at the PSD. Tetramerization of these proteins is required for dendritic spine integrity and to recruit additional proteins to the synapse [Hayashi 2009]. *SHANK2_1* is the largest neuronal isoform of *SHANK2* and is predicted to encode a 1,470 amino acid protein with a Src homology 3 (SH3) domain, a postsynaptic density 95/Discs large/zona occludens-1 homology (PDZ) domain, a proline rich region, and a sterile alpha motif (SAM) domain. Within the proline rich region are binding motifs for HOMER, dynamin-2, and cortactin. The CNVs reported disrupt the PDZ domain and cause a frameshift mutation. The P208S substitution is within the SH3 domain, and the other mutations and variants reported occur either within the proline rich region or outside of the annotated domains. A R462X nonsense mutation is predicted to abrogate the C-terminal region including the SAM domain that is critical for localization at synapses, and a T1127M substitution is within the highly conserved dynamin-2 binding site [Berkel 2010].

Mice have been developed with the genetic deletion of *ProSAP1/Shank2*, and both heterozygotes and homozygotes are viable but with reduced survival rates compared to wild-type littermates. Importantly, these mutants show both aberrant synapses and autistic-like features. Mutants had a reduced number of dendritic spines, higher levels of both the NMDA receptor subunit GluN1 and ProSAP2/Shank3 at the PSD, and an increase in NMDA receptor subunits in the hippocampus and striatum. Intriguingly, ProSAP1/Shank2 is reciprocally upregulated in ProSAP2/Shank3-null mutants. Electrophysiology recording in Shank-2 mutant CA1 pyramidal cells from hippocampal slices revealed decreased field excitatory postsynaptic potentials (fEPSPs), decreased synaptic transmission, and decreased miniature excitatory postsynaptic currents (mEPSCs). Knock-out mice also showed an increase in NMDA/AMPA ratio and an increase in NMDAR-dependent long-term potentiation. In terms of behavior and physiology, mutant mice displayed hindlimb clasping, hyperactivity, reduced digging, extended grooming, difficulty maintaining social contacts, and altered vocalization frequencies [Schmeisser 2012].

Another *Shank2*$^{-/-}$ mouse model harboring a mutation identical to the microdeletion observed in patients, which eliminates both exons 6 and 7, conversely found reduced NMDAR function. Despite this difference, this model also showed ASD-like phenotypes. In this case, restoration of NMDAR function ether by D-cycloserine, an NMDAR partial agonist, or an allosteric modulator of metabotropic glutamate receptor 5 normalized NMDAR currents and social behavior [Won 2012].

Publications:

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