*Late Breaking Chromosomes***SHANK Mutations May Disorder Brain Development**

A hemizygous deletion in region 22q13 has frequently been found in children with neonatal hypotonia, developmental delay, absent to severely delayed speech, autistic behavior, and minor dysmorphic features [Phelan and McDermid, 2012]. This syndrome, termed Phelan McDermid syndrome, has been attributed to mutations of the *SHANK3* gene, which is part of a family of genes, including *SHANK1* and *SHANK2*, that encode post-synaptic scaffold proteins. They consist of one SPN domain, 3 or 4 ankyrin repeats, a Src homology domain 3, a PSD-95/Dlg/ZO-1 domain, a proline-rich region, and a sterile alpha motif [Sheng and Kim, 2000; Leblond et al., 2014]. The SHANK proteins localize to the post-synaptic density, where they connect the actin cytoskeleton to the glutamatergic receptor region and G protein-coupled signaling pathways, and contribute to synapse formation and dendritic spine maturation. In patients with mutations in one allele or hemizygous deletions of *SHANK3*, vermis hypoplasia, striatal dysfunction and altered dendritic spine morphology have been found [Peça et al., 2011; Wang et al., 2011, 2014; Aldinger et al., 2013; Durand et al., 2012]. With in vitro RNAi knockdown experiments, altered regulation of metabotropic glutamate receptor 5 (mGluR5) expression and signaling at synapses was demonstrated [Verpelli et al., 2011]. Hemizygous losses and mutations of *SHANK3* were detected in patients with developmental delay, autism spectrum disorder (ASD) and schizophrenia [Durand et al., 2007; Marshall et al., 2008; Gauthier et al., 2010]. Conversely, duplication of *SHANK3* induces bipolar disorders with progressive loss of skills, attention deficit hyperactivity disorder (ADHD), or episodes of status epilepticus [Denayer et al., 2012; Han et al., 2013]. So, clinical phenotypes, albeit of strikingly dif-

ferent nature, are caused by either loss or duplication of *SHANK3*. Thus, mutations of *SHANK3*, provoking haploinsufficiency or triplosufficiency, are sufficient to elicit a clinical phenotype. Such a dominant effect of mutations suggests that *SHANK3* may be a node in a combinatorial genetic network, as for instance *CNTNAP2* [Poot et al., 2011; Poot, 2015]. It remains conceivable, however, that some mutations of *SHANK3* may alone be sufficient to elicit a clinical disorder. To shed light on these issues and to derive possible phenotype-genotype relationships, a closer examination of a large number of mutations may be a promising approach [Leblond et al., 2014].

Leblond et al. [2014] analyzed copy-number variants (CNVs) in 5,657 ASD patients and 19,163 healthy controls, and ascertained single nucleotide variants (SNVs) in coding sequences of the SHANK genes in 760–2,147 ASD patients and 492–1,090 controls (depending on the gene examined) by performing classical dideoxy sequencing of the 3 SHANK genes. In approximately 1% of patients with ASD, CNVs and gene-truncating mutations were found. SNVs in *SHANK1* (0.04%) were only found in male patients with normal IQ and ASD, while SNVs in *SHANK2* were detected in 0.17% of patients with ASD and mild intellectual disability. In contrast, SNVs in *SHANK3* were found in 0.69% of patients with ASD and in up to 2.12% of the ASD cases, moderate to profound intellectual disability was found. The frequencies of CNVs and SNVs of *SHANK2* and *SHANK3* were significantly higher than in healthy controls. In previous exome-sequencing efforts, only 1 de novo SNV in *SHANK2* and no truncating coding-sequence variants in *SHANK1* and *SHANK3* have been found [Neale et al., 2012; O’Roak et al., 2012; Sanders et al., 2012]. This may be due to the poor mutation detection rate in stretches of GC base pairs by the current exome-sequencing technology. SNVs were

found in all domains of the SHANK proteins, with no difference in distribution between ASD patients and controls. To assess the possible pathogenicity of SNVs, their respective Genomic Evolution Rate Profile and Grantham matrix scores were determined [Grantham, 1974; Cooper et al., 2005]. There was no significant difference between ASD patients and healthy controls regarding these scores for the SNVs examined. However, in ASD patients, but not in healthy controls, in-frame deletions presumed to remove several amino acids in the SHANK2 and SHANK3 proteins were found.

Given the significantly high frequencies of variants in SHANK2 and SHANK3 in ASD patients versus healthy controls and the impact of such variants on neuronal development, a molecular hypothesis to explain these findings is needed. Conceivably, certain SHANK2 and SHANK3 variants may by themselves be sufficient to elicit ASD or ASD with moderate to severe ID, respectively. On the other hand, a polygenic model of gene action should also be considered. Leblond et al. [2012] found 2 patients with *SHANK2* mutations showing an additional gain of the nicotinic receptor gene *CHRNA7* and in 1 patient a loss of the *CYFIP1* gene. This study supports a model of epistatic interactions of genes in distinct biological pathways, which upon disruption may lead to ASD.

In mice, deletion of both *Shank2* alleles results in an early, brain-region-specific upregulation of ionotropic glutamate receptors at the synapse and increased levels of ProSAP2/Shank3 [Schmeisser et al., 2012]. *Shank2*^{-/-} mutant mice have fewer dendritic spines and show reduced basal synaptic transmission, enhanced N-methyl-D-aspartate receptor (NMDAR)-mediated excitatory

currents, are extremely hyperactive and display profound autistic-like behavioral alterations including repetitive grooming as well as abnormalities in vocal and social behaviors [Schmeisser et al., 2012]. Comparing ProSAP1/Shank2^{-/-} and ProSAP2/Shank3αβ^{-/-} mice, the authors found that different abnormalities in synaptic glutamate receptor expression can cause distinct alterations in social interactions and communication. Treatment of *Shank2*^{-/-} mice with a positive allosteric modulator of metabotropic glutamate receptor 5 (mGluR5) normalizes NMDAR function and markedly enhances social interaction [Won et al., 2012]. These results suggest that reduced NMDAR function, resulting from deletion of both alleles of *Shank2* in mice, may contribute to the development of ASD-like phenotypes, and that mGluR modulation of NMDARs may offer a potential approach to treat ASD [Won et al., 2012].

Both the data on transgenic mice and the earlier study of Leblond et al. [2012] carry important implications for medical genetic practice. First, screening for *SHANK2* and *SHANK3* variants by classical dideoxy sequencing should be included in the laboratory workup of all ASD patients. Second, once such a variant has been detected, genome-wide CNV analysis should be done in order to search for other mutated genes so that a complete as possible picture of the mutational landscape in the individual ASD patient can be obtained [Leblond et al., 2012; Poot et al., 2011]. Third, with this information we may be able to label selected patients as ‘SHANK-type’ ASD and may then consider therapies that match their underlying synaptopathic phenotype [Schmeisser et al., 2012; Poot, 2013].

Martin Poot

References

- Aldinger KA, Kogan J, Kimonis V, Fernandez B, Horn D, et al: Cerebellar and posterior fossa malformations in patients with autism-associated chromosome 22q13 terminal deletion. *Am J Med Genet A* 161A:131–136 (2013).
- Cooper GM, Stone EA, Asimenos G; NISC Comparative Sequencing Program, Green ED, et al: Distribution and intensity of constraint in mammalian genomic sequence. *Genome Res* 15:901–913 (2005).
- Denayer A, Van Esch H, de Ravel T, Frijns JP, Van Buggenhout G, et al: Neuropsychopathology in 7 patients with the 22q13 Deletion Syndrome: presence of bipolar disorder and progressive loss of skills. *Mol Syndromol* 3:14–20 (2012).
- Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, et al: Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nat Genet* 39:25–27 (2007).
- Durand CM, Perroy J, Loll F, Perrais D, Fagni L, et al: *SHANK3* mutations identified in autism lead to modification of dendritic spine morphology via an actin-dependent mechanism. *Mol Psychiatry* 17:71–84 (2012).
- Gauthier J, Champagne N, Lafrenière RG, Xiong L, Spiegelman D, et al: De novo mutations in the gene encoding the synaptic scaffolding protein SHANK3 in patients ascertained for schizophrenia. *Proc Natl Acad Sci USA* 107: 7863–7868 (2010).
- Grantham R: Amino acid difference formula to help explain protein evolution. *Science* 185: 862–864 (1974).
- Han K, Holder JL Jr, Schaaf CP, Lu H, Chen H, et al: *SHANK3* overexpression causes manic-like behaviour with unique pharmacogenetic properties. *Nature* 503:72–77 (2013).
- Leblond CS, Heinrich J, Delorme R, Proepper C, Betancur C, et al: Genetic and functional analyses of *SHANK2* mutations suggest a multiple hit model of autism spectrum disorders. *PLoS Genet* 8:e1002521 (2012).

- Leblond CS, Nava C, Polge A, Gauthier J, Huguet G, et al: Meta-analysis of *SHANK* mutations in autism spectrum disorders: a gradient of severity in cognitive impairments. *PLoS Genet* 10:e1004580 (2014).
- Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, et al: Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet* 82:477–488 (2008).
- Neale BM, Kou Y, Liu L, Ma'ayan A, Samocha KE, et al: Patterns and rates of exonic de novo mutations in autism spectrum disorders. *Nature* 485:242–245 (2012).
- O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, et al: Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature* 485:246–250 (2012).
- Peça J, Feliciano C, Ting JT, Wang W, Wells MF, et al: *Shank3* mutant mice display autistic-like behaviours and striatal dysfunction. *Nature* 472:437–442 (2011).
- Phelan K, McDermid HE: The 22q13.3 Deletion Syndrome (Phelan-McDermid Syndrome). *Mol Syndromol* 2:186–201 (2012).
- Poot M: Towards identification of individual etiologies by resolving genomic and biological conundrums in patients with autism spectrum disorders. *Mol Syndromol* 4:213–226 (2013).
- Poot M: Connecting the CNTNAP2 networks with neurodevelopmental disorders. *Mol Syndromol* (2015, in press).
- Poot M, van der Smagt JJ, Brilstra EH, Bourgeron T: Disentangling the myriad genomics of complex disorders, specifically focusing on autism, epilepsy, and schizophrenia. *Cytogenet Genome Res* 135: 228–240 (2011).
- Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, et al: De novo mutations revealed by whole-exome sequencing are strongly associated with autism. *Nature* 485: 237–241 (2012).
- Schmeisser MJ, Ey E, Wegener S, Bockmann J, Stempel AV, et al: Autistic-like behaviours and hyperactivity in mice lacking ProSAP1/Shank2. *Nature* 486:256–260 (2012).
- Sheng M, Kim E: The Shank family of scaffold proteins. *J Cell Sci* 113(Pt 11):1851–1856 (2000).
- Verpelli C, Dvoretzskova E, Vicidomini C, Rossi F, Chiappalone M, et al: Importance of Shank3 protein in regulating metabotropic glutamate receptor 5 (mGluR5) expression and signaling at synapses. *J Biol Chem* 286:34839–34850 (2011).
- Wang X, McCoy PA, Rodriguiz RM, Pan Y, Je HS, et al: Synaptic dysfunction and abnormal behaviors in mice lacking major isoforms of *Shank3*. *Hum Mol Genet* 20:3093–3108 (2011).
- Wang X, Xu Q, Bey AL, Lee Y, Jiang YH: Transcriptional and functional complexity of *Shank3* provides a molecular framework to understand the phenotypic heterogeneity of SHANK3 causing autism and *Shank3* mutant mice. *Mol Autism* 5:30 (2014).
- Won H, Lee HR, Gee HY, Mah W, Kim JI, et al: Autistic-like social behaviour in *Shank2*-mutant mice improved by restoring NMDA receptor function. *Nature* 486:261–265 (2012).