*Late Breaking Chromosomes***What Is in a Single Exon or Intron of *AUTS2*?**

The autism susceptibility candidate 2 (*AUTS2*) gene in region 7q11.2 came to the attention of medical geneticists after a t(7;20)(q11.2;p11.2) translocation was found in a monozygotic twin with autism [Sultana et al., 2002]. This gene received further attention when de novo balanced translocations, truncating *AUTS2*, were detected in 3 unrelated mentally disabled patients [Kalscheuer et al., 2007], and thirdly, a paracentric inversion 7q11.22q35 involving both *AUTS2* and *CNTNAP2* was found in another patient with autism [Bakkaloglu et al., 2008]. Summarizing the data on structural disruptions of *AUTS2*, Poot et al. [2011] suggested that disruptions causing truncation beyond exon 6 of *AUTS2* may cause milder intellectual delay (mental retardation) phenotypes, while hemizygous losses of exon 6 of *AUTS2* may precipitate either profound to severe intellectual delay (mental retardation) [Kalscheuer et al., 2007] or may result in pervasive developmental disorder, not otherwise specified, or even autism [Kalscheuer et al., 2007; Huang et al., 2010]. Three recent publications have now sharpened our insights into the effects of disruptions of *AUTS2* down to the level of a single exon or intron.

Using a specially designed exon-targeted array, Nagamani et al. [2013] found small copy number variations in 4 patients, ranging in size between 133 and 319 kb disrupting *AUTS2*. Two patients with autism have duplications involving only exon 5, whereas 2 other patients without the diagnosis of autism have deletions that re-

moved multiple, more distal exons. All 4 patients showed developmental delay, whereas the 2 patients with a duplication of exon 5 were discordant for a seizure phenotype. In 2 out of 517 patients with idiopathic generalized or focal epilepsies, *AUTS2* losses were identified: one in a patient with juvenile myoclonic epilepsy and one in a patient with unclassified non-lesional epilepsy with features of atypical benign partial epilepsy [Mefford et al., 2010]. These latter findings broaden the scope of *AUTS2* to epilepsy and maybe other neurobehavioral disorders.

In a large collaborative study, which covered 49,684 individuals, Beunders et al. [2013] found 24 patients with disruptions of *AUTS2*, of which 17 appeared to share a complex syndrome including intellectual delay, autism, a short stature, microcephaly, cerebral palsy, and facial dysmorphisms. Patients with the largest number of phenotypic features shared losses from exon 6 on until the 3' end of the gene, while patients with more proximal losses showed less features. In 8 out of 11 patients in which both parents were available for genetic testing, the *AUTS2* losses had occurred de novo, while 3 patients inherited the *AUTS2* deletion from an unaffected parent (one case) or an affected parent (2 cases). In 6 families, the inheritance status of the *AUTS2* losses could not be fully resolved. Of the 10 patients with an intragenic loss (excluding the first and last exon), 4 patients carried a loss predicted to cause a frameshift, whereas the other 6 individuals carried in-frame losses. Three patients carried losses that involved at least one downstream gene (*WBSCR17*).

Since ENSEMBL annotation of *AUTS2* predicts several splice isoforms, Beunders et al. [2013] looked for the presence of alternative isoforms of *AUTS2* in human brain mRNA. Using 5' RACE, they identified a short 30-bp *AUTS2* mRNA variant starting in the middle of exon 9. Suppression of *auts2* in zebrafish embryos caused microcephaly. Either the full length or the C-terminal isoform of *AUTS2* completely rescued this phenotype. This indicates that the C-terminal alternative splice variant of *AUTS2* may be involved in the dysmorphic phenotypes of patients with C-terminal losses.

The sequence context surrounding *AUTS2* is one of the least conserved between modern humans and Neanderthals [Green et al., 2010]. This is consistent with a possible role of *AUTS2* in neurological disease, in particular relating to human-specific behavior. Since changes in the *AUTS2* locus are associated with autism and other neurological disorders, including attention deficit hyperactivity disorder, epilepsy, dyslexia, motor delay, language delay, visual impairment, and microcephaly (see above), Oksenberg et al. [2013] set out to functionally characterize *auts2* by using zebrafish morpholinos. Knocking down this gene leads to an overall stunted developmental phenotype, including a smaller head and body with re-

duced body movements. The morphant fish showed a reduction in developing a midbrain, sensory and motor neurons. Using both zebrafish and mouse transgenic enhancer assays, the authors identified 3 functional enhancers within an ASD-associated deletion and 6 brain enhancers in regions associated with human-specific evolution. These findings indicate that *AUTS2* is important for neuronal development, and that several functional enhancers within this locus may be associated with neurodevelopmental disease and human-specific evolution. *AUTS2* may, thus, join genes like *CNTNAP2* and *FOXP2*, which have been related to pleiotropic neurobehavioral phenotypes and being part of complex regulatory networks in which each and every exon and even intronic sequences count [Poot et al., 2010, 2011; Vernes et al., 2011; Graham and Fisher, 2013].

The recent flurry of studies relating to *AUTS2* have not only broadened and sharpened our insights into the impact of a single exon, but also of what may lay hidden in the introns of genes. Most importantly, these studies indicate the need for more elaborate and thorough functional studies in model organisms.

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