Disconnecting *CNTNAP2*

Genomic rearrangements and copy number variations (CNVs) have implicated the contactin-associated protein-like 2 gene (*CNTNAP2*) in a variety of neurodevelopmental disorders, such as Gilles de la Tourette syndrome, intellectual disability, obsessive compulsive disorder, cortical dysplasia-focal epilepsy syndrome, autism, schizophrenia, Pitt-Hopkins syndrome, and attention deficit hyperactivity disorder [Rodenas-Cuadrado et al., 2014; Poot, 2015]. *CNTNAP2* spans 2.3 Mb genomic DNA and encodes a full-length transcript of 24 exons. The *CNTNAP2*-encoded CASPR2 protein of 1,331 residues consists of a signal peptide, an F5/8 discoidin/neuropilin homology domain, 4 laminin G-binding domains, 2 epidermal growth factor-like domains, a fibrinogen-like region, a transmembrane domain, and a post-synaptic density interaction domain. Losses of a single allele of a gene may exert phenotypic effects if that gene is part of either a gene network or if the protein it encodes interacts with other proteins. In the latter case, such disruptions will affect the stoichiometry among members of macromolecular complexes, such as membrane receptors, or signaling pathways [Poot et al., 2011; Birchler and Veitia, 2012]. In addition, the *CNTNAP2*-encoded CASPR2 protein may serve as a 'bridge' across synapses between neurons by binding proteins to another cell. Several recent reports have indicated physical interactions of the CASPR2 intracellular domain with *ADAM22*, *LGII*, *KCNA1* (Kv1.1 channels), *GPR37* and of the extracellular domains with *GRIA2*, *DLG1*, *DLG4*, and *CNTN1* [Chen et al., 2015; Tanabe et al., 2015; Rubio-Marrero et al., 2016]. An association study and a survey of mutations in 2 large cohorts of patients with autism indicated a possible interaction of *CNTNAP2* with *CNTN6* [Poot, 2014; Mercati et al., 2016]. Yet to what extent and by which mechanism(s) perturbation of these interactions may

contribute to the variety of clinical phenotypes among patients with *CNTNAP2* disruptions remains unclear.

Several publications indicating that a double hit, i.e., losing both alleles of *CNTNAP2*, provokes a specific clinical disorder and may provide insights into the remarkable clinical variability of *CNTNAP2* disruptions [Strauss et al., 2006; Zweier et al., 2009; Watson et al., 2014; Rodenas-Cuadrado et al., 2016]. Patients with homozygous losses of *CNTNAP2* present with a syndrome involving severe intellectual disability, early-onset drug-resistant epilepsy with concomitant regression of language, communicative impairments and autistic features, albeit with some variability among these phenotypes. On the basis of the genomic losses, a truncated protein that no longer contains the membrane-anchoring domain of CASPR2 would be predicted in 3 of these cases [Strauss et al., 2006; Watson et al., 2014; Rodenas-Cuadrado et al., 2016]. In the fourth case, the membrane-anchoring domain would be retained, but 2 of the N-terminal laminin G-binding domains would be lost [Zweier et al., 2009]. *CNTNAP2* is weakly transcribed in peripheral blood mononuclear cells or cultured fibroblasts. Therefore, the effects of these homozygous losses on *CNTNAP2*-transcription have not been rigorously investigated in these patients. Since both *CNTNAP2* alleles have been lost, the cell cannot generate a full-length transcript. Therefore, either a truncated mRNA and consequently a truncated protein, or complete absence of any *CNTNAP2* transcripts should account for the clinical phenotype of these patients. Without information on the putative expression of these mutated *CNTNAP2* alleles, one cannot decide between these 2 inferences.

In a case with an inherited heterozygous loss of exons 14 and 15 of *CNTNAP2*, inducible pluripotent stem cells were generated, which were subsequently made to differentiate into neurons [Lee et al., 2015]. The authors

found that in these cells in their patient the mutated allele was overexpressed, while in the healthy carrier father the full-length transcript was the predominant one [Lee et al., 2015]. The mutated allele would probably be translated into a protein lacking the C-terminal epidermal growth factor-like domain. The authors propose 3 hypotheses to explain their findings. First, the carrier father has protective alleles that were not transmitted to the affected daughter. Second, the affected daughter has additional deleterious alleles, which had either arisen de novo or were inherited from her mother, but not from her father. Third, *CNTNAP2* alleles with structural deletions may experience incomplete penetrance and variable expressivity as a functional consequence of variable levels of the mutated *CNTNAP2* gene being expressed [Lee et al., 2015]. The latter has recently been suggested as a possible contribution to the observed variability among patients with intragenic *CNTNAP2* losses [Poot, 2015]. Preferential expression of either the mutant or the wild-type allele is another possible explanation for the incomplete pene-

trance of disease risk genes in general [Malhotra and Sebat, 2012]. Transcriptome sequencing of differentiating neurons derived from cultures of inducible pluripotent stem cells from patients with autism and schizophrenia revealed an enrichment of candidate genes for these disorders among those that showed allele-biased expression [Lin et al., 2012]. Such mutated alleles will encode aberrant forms of CASPR2, which may engage in distinct patterns of physical interaction in patients relative to healthy individuals. In addition, CASPR2 may bind to different proteins in the various areas of the brain where it is expressed. Losing one or several of these interactions may then provoke any of the many clinical phenotypes which are currently associated with heterozygous disruptions of the *CNTNAP2* gene. These recent studies have provided novel insights into the possible mechanisms by which *CNTNAP2* may be involved in a variety of neurodevelopmental disorders and may spur further research.

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