***HNRNPU*: Key to Neurodevelopmental Disorders such as Intellectual Delay, Epilepsy, and Autism**

Children with neurodevelopmental disorders, consisting of combinations of functional impairments and structural anomalies, often do not fit any known syndrome and seem to emerge “out of the blue” in families without any history of genetic disease. The current paradigm in medical genetics assumes that such isolated and seemingly unique cases may have resulted from either a *de novo* copy number variation (CNV) or a single nucleotide variant (SNV). Therefore, the genomes of such patients and their parents are investigated by either array-CGH or by whole-genome sequencing. Initially, terminal deletions of chromosome region 1q44 were found in 2 unrelated patients with a thin corpus callosum and a Dandy-Walker complex, which is a structural brain anomaly consisting of posterior fossa cystic malformations and cerebellar anomalies [van Bever et al., 2005; Poot et al., 2007]. The smaller of the 2 deletions had a breakpoint in the olfactory receptor gene cluster in chromosome region 1q44 and comprised 17 annotated genes of which several were implicated in brain development [Poot et al., 2007]. Subsequently, array CGH of a series of children with microcephaly and agenesis of the corpus callosum revealed partially overlapping deletions in chromosome band 1q44, which shared a critical region of 3.5 Mb [Boland et al., 2007]. In one patient, a balanced reciprocal translocation, t(1;13)(q44;q32), mapped 20 kb upstream of *AKT3*, a serine-threonine kinase, which in mice, regulates overall brain size and the thickness of the corpus callosum during development. The *AKT3* gene was deleted in the case of van Bever et al. [2005], but unaffected in the patient described by Poot et al. [2007], and in an indepen-

dent series of patients analyzed by van Bon et al. [2008]. The findings of Poot et al. [2007] and by van Bon et al. [2008] were not consistent with mere hemizygosity for *AKT3* as an explanation for features such as microcephaly, polymicrogyria, agenesis of the corpus callosum, and vermis hypoplasia in patients with deletions of the 1q44 region [Poot et al., 2008]. However, a combination of a hemizygous loss of and a mutation in the *AKT3* gene could be pathogenic and may explain the variability in the corpus callosum phenotype among patients with 1q44 losses [Poot et al., 2011]. Sequencing *AKT3* in 66 patients with an abnormal corpus callosum did not reveal any mutations [Boland et al., 2007; van Bon et al., 2008]. In contrast, *AKT3* mutations have been described in 2 sporadic cortical malformation disorders: the hemimegalencephaly and the megalencephaly-capillary malformation with megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndromes [Lee et al., 2012; Rivière et al., 2012]. These findings indicate a role of *AKT3* in cortical development, well beyond the realm of 1q44 deletions [Poot et al., 2008], but leave open the question of what other gene or genes in the 1q44 region may also be involved in controlling brain development.

Genotype-phenotype relationships for CNVs encompassing multiple genes can be resolved by identifying candidate genes through systematic mapping of regions with overlapping phenotypes. Caliebe et al. [2010] studied 4 patients with overlapping deletions in region 1q44, who showed developmental delays (in particular of expressive speech), seizures, hypotonia, and CNS anomalies, including abnormal thickness of the corpus callosum in 3 out of

4 patients. Oligonucleotide and SNP-array-based profiling revealed that 3 of these patients shared a 0.440-Mb interstitial deletion, which did not overlap with the previously published consensus regions of 1q44 deletions. In all 4 patients, *AKT3* and *ZNF238*, 2 previously proposed candidate genes for microcephaly and agenesis of the corpus callosum, were unaffected [Boland et al., 2007; Caliebe et al., 2010]. In contrast, the 0.440-Mb shared interstitial deletion of the 4 patients encompassed the *FAM36A*, *HNRNPU*, *EFCAB2*, and *KIF26B* genes [Caliebe et al., 2010]. The authors argue that *HNRNPU*, which is involved in the regulation of embryonic brain development, may be a plausible candidate gene for the combination of developmental delay, speech delay, hypotonia, hypo- or agenesis of the corpus callosum, and seizures in patients with 1q44 deletions. Since only 3 of the 4 patients showed an abnormal thickness of the corpus callosum, additional mechanisms, such as unmasking of hemizygous mutations, position effects, and possible interactions with other loci, have to be considered. Ballif et al. [2012] analyzed 22 individuals by high-resolution oligonucleotide microarray-based comparative genomic hybridization and proposed 3 critical regions as well as candidate genes for microcephaly, abnormal corpus callosum thickness, and seizures within region 1q44. Three cases with microcephaly had overlapping or intragenic deletions of *AKT3*, implicating haploinsufficiency of this gene as a cause of microcephaly. The shared critical region for an abnormal corpus callosum contained only *ZNF238*, a transcriptional and chromatin regulator highly expressed in the developing and adult brain. Finally, the patients with seizures shared a region containing 3 genes (*FAM36A*, *C1ORF199*, and *HNRNPU*). The authors indicate that the phenotypic variability among their patients suggests that mechanisms such as variable expressivity, incomplete penetrance, position effects, or multigenic factors may contribute to the phenotypic complexity of some cases [Ballif et al., 2012].

Studying 17 patients with 1q43q44 microdeletions, 4 with *ZBTB18* mutations and 7 with *HNRNPU* mutations, Depienne et al. [2017] concluded that *AKT3* haploinsufficiency is the main driver for microcephaly. *HNRNPU* alterations mostly lead to epilepsy, and to some degree of intellectual disability, and *ZBTB18* deletions or mutations were associated with variable corpus callosum anomalies, albeit with incomplete penetrance. *ZBTB18* may also contribute to microcephaly and *HNRNPU* to a thin corpus callosum but with a lower penetrance. The authors suggest that co-deletion of multiple genes in cases with a contiguous gene syndrome may have additive effects.

The case for *HNRNPU* as a key gene in brain development was further strengthened by mapping genetic loci for variation in neuroanatomical structures in recombinant inbred mouse strains, which allows identifying eQTLs for complex developmental phenotypes. Poot et al. [2011] found a consistent association between *HNRNPU* genetic variation and corpus callosum integrity in both mice and humans. This suggests that neurodevelopmental processes, such as those underlying corpus callosum development, are evolutionary conserved across mammalian species [Poot et al., 2011].

Sequencing *HNRNPU* of 191 patients with intellectual delay, of whom 112 were diploid for their entire genome, Thierry et al. [2012] did not find any potentially pathogenic *HNRNPU* mutations. This led Poot and Kas [2013] to suggest that hemizygous deletion of the *HNRNPU-AS1* gene, which is located within the introns of *HNRNPU*, instead may affect both its own expression level and that of *HNRNPU*. In such cases, an imbalance in *HNRNPU* expression levels, rather than gene mutations, may be responsible for the intellectual delay phenotype of patients with hemizygous losses of *HNRNPU*.

Yates et al. [2017] and Leduc et al. [2017] took an inverse approach: they compiled shared phenotypes of patients with de novo *HNRNPU* mutations detected by sequencing patient-parent trios. Within the Wellcome Trust Deciphering Developmental Disorders study, they found 7 patients with dysmorphic features, including prominent eyebrows, long palpebral fissures, overhanging columella, and a thin upper lip, as well as developmental delay, intellectual disability, and seizures during febrile episodes from early childhood on [Yates et al., 2017]. In an independent cohort, 4 patients with de novo heterozygous loss-of-function mutations of *HNRNPU* shared seizures, global developmental delay, intellectual disability, variable neurological regression, behavior issues, and dysmorphic facial features [Leduc et al., 2017]. No cardiac and renal abnormalities were detected. In a third cohort, 6 patients with de novo heterozygous loss-of-function mutations of *HNRNPU* shared early-onset seizures, severe intellectual delay, severe speech impairment, and hypotonia [Bramswig et al., 2017]. Cardiac and renal abnormalities were found in 4 and 3 of these patients, respectively. The discovery of postzygotic mutations in a cohort of 5,947 patient-parent trios of families with autism spectrum disorder further enlarged the phenotypic spectrum associated with de novo *HNRNPU* mutations [Lim et al., 2017].

To understand the bewildering phenotypic variability associated with CNVs and SNVs affecting *HNRNPU*, one

should take into account that CNVs often cause a broader phenotypic spectrum than SNVs do [Cai et al., 2003; Poot and Haaf, 2015]. First, CNVs may alter the copy number of a single or several genes at once in a contiguous gene syndrome. Second, a CNV may disrupt a gene or the topologically associated domains (TADs) of which it is part or that are located in close vicinity to the breakpoints of the CNV [Lupiáñez et al., 2016; Loviglio et al., 2017; Spielmann et al., 2018].

Recent evidence regarding the function(s) of *HNRNPU* may point towards a few novel mechanisms leading to the phenotypic variability associated with CNVs and SNVs involving this gene [Geuens et al., 2016; Attig et al., 2018; Zhang et al., 2018]. The *HNRNPU*-encoded heterogeneous nuclear ribonucleoprotein U is involved in transcription and alternative splicing; it is needed for the accumulation of the long noncoding RNA Xist on chromosome X [Hasegawa et al., 2010; Bi et al., 2013; Vu et al., 2013; Geuens et al., 2016]. Thus, the HNRPU protein epigenetically inactivates 1 of the 2 female X chromosomes to equalize gene expression with male mammals. A chromosome conformation capture (3C) study showed that HNRNPU is involved in the 3D organization of the genome [Zhang et al., 2018]. Loss of the HNRPU protein may result in either compartment switching within the genome or reduction in the strength of boundaries between TADs. These events affect the control of chromatin loop formation and may alter gene expression, even at a distance from the HNRNPU-binding site given the size of some TADs [Lupiáñez et al., 2016; Spielmann et al., 2018]. In addition, HNRNPU mainly binds to actively tran-

scribed chromatin, in particular to CTCF or RAD21 sites. Depending on the amount of HNRNPU protein available in the cell nucleus, these sites will be differently occupied and consequently their target genes are more or less expressed. Thus, a CNV altering the *HNRNPU* gene dosage may affect expression of a wide range of downstream proteins and eventually cause distinct spectra of phenotypes. Intragenic SNVs, on the other hand, may change the binding affinities to cognate HNRNPU targets, such as CTCF and RAD21 sites, and allow the protein to bind to other, illegitimate, targets or abolish target binding altogether. Recently, the HNRNPU protein has been shown to bind to long interspersed nuclear elements of the L2 type in antisense orientation [Attig et al., 2018]. Binding of HNRNPU and other of RNA-binding proteins block long introns and thus allow the transcription machinery to properly identify exons during the splicing process needed to form proper mRNAs. Conceivably, a lowered level of HNRNPU protein may lead to spurious missplicing. Probably CNVs and SNVs of the *HNRNPU* gene may produce distinct phenotypic outcomes. In view of the plethora of possibly pathogenic molecular mechanisms, there is a clear need for in vitro and in vivo functional studies to assess the effects of CNVs and the different SNVs in HNRNPU, as has been done for CNTNAP2 SNVs [Brumback et al., 2017; Scott et al., 2017; Canali et al., 2018; Gao et al., 2018; Liska et al., 2018; Vogt et al., 2018; Zerbi et al., 2018]. The outcome of such studies may help us understand the phenotypic variability in patients with CNVs and SNVs affecting *HNRNPU*.

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