Syndromes Hidden within the 16p11.2 Deletion Region

Copy number variations (CNVs) are a form of structural genome variations by which a stretch of chromatin between 2 breakpoints on the same chromosome arm is either lost or duplicated. CNVs larger than 100 kb arise de novo in the general population at an estimated rate of about 1.2×10^{-2} CNVs per meiosis [Itsara et al., 2010]. In roughly 14–18% of the children with developmental delay, a CNV larger than 400 kb may be phenotypically significant [Cooper et al., 2011; Hochstenbach et al., 2011]. CNVs occur more frequently than germline single nucleotide variations (SNVs), and they affect more nucleotides than SNVs [Stankiewicz and Lupski, 2010; Campbell and Eichler, 2013]. CNVs may either be flanked by unique DNA sequences or by segmental duplications. While the first occur spontaneously and are unique in size and breakpoint positions, the second type of CNVs are recurrent and of a fixed size. The segmental duplications flanking recurrent CNVs are remnants of chromosomal evolution, which render their loci inherently unstable [Eichler et al., 2001; Samonte and Eichler, 2002; Bailey and Eichler, 2006].

Although recurrent CNVs occur at higher frequency in patient cohorts than in the general population, some were found in both children with developmental disorders and their healthy parents [Girirajan et al., 2010; Poot et al., 2010]. This poses particular challenges in gauging their potential phenotypic effects. Girirajan and

Eichler [2010] proposed that such CNVs may interact with additional rare de novo or transmitted alleles to cause complex genetic disorders. Such a constellation may apply to some families of children with autism spectrum disorder (ASD) [Bucan et al., 2009; Salyakina et al., 2011; van Daalen et al., 2011; Poot, 2013]. Alternatively, an inherited or de novo deletion on one chromosome may be paired with a deletion or a gene mutation in the same region on the other transmitted chromosome. Thus, the deletion may “unmask” an inherited or de novo gene mutation or deletion [Flipsen-ten Berg et al., 2007; Hochstenbach et al., 2012; Poot and Haaf, 2015]. A case in point is a girl with attention deficit-hyperactivity disorder and cognitive impairments as well as a de novo heterozygous 600-kb deletion in the 16p11.2 region, which unmasks a heterozygous 2-bp deletion in the *CORO1A* gene, inherited from the unaffected father [Shiow et al., 2009].

However, upon examination of CNVs within the 16p11.2 region, additional potentially pathogenic mechanisms of CNVs may become apparent. By array comparative genomic hybridization, Kumar et al. [2008] identified a recurrent 16p11.2 deletion in 4 out of 712 probands (0.6%) with ASD and 0 in 837 controls, and Marshall et al. [2008] detected this deletion in 4 out of 427 families with ASD patients and none in 1,652 controls [Kumar et al., 2008; Marshall et al., 2008]. Eichler and Zimmerman [2008] noted that this recurrent CNV contained at least

25 genes or transcripts, some of which may cause a phenotypic effect if their dosage is altered. Thus, this recurrent CNV likely represents a contiguous gene syndrome.

Candidate genes for the individual phenotypes associated with a CNV can be identified by comparing rare cases with overlapping CNVs. Such dissection of CNVs into regions with shared phenotypes has allowed pinpointing *HNRNPU* as a gene for epilepsy within the 1q44 region [Caliebe et al., 2010; Ballif et al., 2012; Depienne et al., 2017]. In a cohort of 300 patients with severe early-onset obesity, of whom 143 also had developmental delay, 5 patients with overlapping deletions at chromosome 16p11.2 were found, and 2 in 7,366 controls [Bochukova et al., 2010]. In 3 patients, the deletion cosegregated with severe obesity, while the other 2 patients harbored a larger de novo 16p11.2 deletion, which extended beyond the 593-kb deletion that was previously associated with ASD and intellectual disability. Both of these patients also showed mild developmental delay in addition to severe obesity. All 5 of the 16p11.2 deletions encompassed a 200-kb recurrent CNV interval, which included *SH2B1*, a gene known to be involved in leptin and insulin signaling [Bachmann-Gagescu et al., 2010]. The 16p11.2 deletion carriers exhibited hyperphagia and severe insulin resistance disproportionate for the degree of obesity. Walters et al. [2010] confirmed these findings on a highly penetrant form of obesity, initially found in 31 patients heterozygous for 16p11.2 deletions of at least 593 kb, who were investigated because of cognitive deficits among other phenotypes. In GWAS data on 16,053 individuals from 8 European cohorts, 19 similar deletions were identified [Walters et al., 2010]. These deletions were absent from healthy nonobese controls and accounted for 0.7% of the morbid obesity cases. In 28 cohorts totaling more than 30,000 cases referred for developmental or intellectual delay, 138 carriers of a duplication in region 16p11.2 of at least 593 kb with reduced postnatal weight and body mass index were found [Jacquemont et al., 2011]. Thus, the phenotypes of the duplication carriers seem to mirror those of the deletion carriers. The authors suggested that severe obesity and being underweight may have common etiologies, possibly through contrasting effects on energy balance. On the other hand, speech delay, resulting from impairments in the basic mechanisms of speech motor control, and behavioral problems, such as ASD and attention-deficit hyperactivity disorder, are the predominant features in individuals with developmental delay and 16p11.2 deletions and duplications [Rosenfeld et al., 2010; Shinawi et al., 2010; Demopoulos et al., 2018]. Both deletion and duplication carriers show a lowered IQ [Hip-

polyte et al., 2016]. While deletion carriers exhibit severe impairments of language and verbal skills, duplication carriers, in contrast, outperform siblings with the same IQ level for their language and verbal abilities [Hippolyte et al., 2016]. These findings underscore the importance of rare variants with a strong effect, albeit they show incomplete penetrance and variable expressivity [Rosenfeld et al., 2010; Shinawi et al., 2010]. The latter may relate to the maternal bias for the origin of deletions (89.4% of the cases) and a maternal transmission bias for secondary deletions (69.5% of the cases) [Duyzend et al., 2016].

To elucidate the complex genotype-phenotype relationships with respect to CNVs of the 16p11.2 region, mouse models on a C57BL/6N inbred genetic background were created [Arbogast et al., 2016]. Mice carrying a heterozygous deletion (*Del/+*) of the region from the *Sult1a1* to the *Spn* gene, which is homologous to the human 16p11.2 593-kbp locus between breakpoints 4 and 5 (BP4–BP5), showed reduced weight and impaired adipogenesis, hyperactivity, repetitive behaviors, and memory deficits. In contrast, *Dup/+* mice showed opposite phenotypes. While the behavioral impact of altered genetic dosage of the 16p11.2 region was similar in mice and humans (e.g., activity and memory alterations), the metabolic defects were opposite. Adult *Del/+* mice are lean in comparison to the obese humans, and the *Dup/+* mice are overweight in comparison to the underweight humans. Moreover, different patterns of aberrant behavior and structural brain abnormalities were found in engineered mice with different genetic background [Horev et al., 2011; Portman et al., 2014; Arbogast et al., 2016]. Thus, the authors suggested that dosage imbalance at the 16p11.2 locus perturbs the expression of modifiers outside this CNV, which can modulate the penetrance, expressivity, and direction of effects in both humans and mice [Arbogast et al., 2016].

In humans, the 16p11.2 region consists of a distal BP2–BP3 interval of 220 kb and a proximal BP4–BP5 interval of 593 kb. The findings in the mouse were extended by investigation of a cohort of 137 unrelated human deletion and reciprocal duplication carriers of the distal with 4C-seq, FISH, Hi-C sequencing, and expression analyses for the affected genes [Loviglio et al., 2017]. This study confirmed that 16p11.2 rearrangements are associated with ASD and the mirror phenotypes of obesity/underweight and macrocephaly/microcephaly. Such phenotypes were previously associated with rearrangements of the nonoverlapping proximal 16p11.2 BP4–BP5 interval of 593 kb. Both CNV-prone regions at 16p11.2 are reciprocally engaged in complex chromatin looping. To iden-

tify pathways that are perturbed when the dosage of this region is modified, the authors determined the chromosomal contacts of genes mapping within the 16p11.2 BP4–BP5 interval by chromosome conformation capture. The promoters of 2 of the drivers of the 16p11.2 phenotype, *MVP* and *MAPK3*, engage in long-range chromatin interactions with *PTEN* and *CHD1L*, respectively. The MVP protein regulates the intracellular localization of PTEN, which in turn antagonizes the PI3K/AKT and Ras/MAPK signaling pathways. Both *PTEN* germline mutations in humans and targeted inactivation in mice were associated with a macrocephaly/autism syndrome (OMIM 605309). On the other hand, germline mutations in the Ras/MAPK pathway cause a set of syndromes, termed RASopathies, which among other phenotypes affect social interaction. This study demonstrated that expression of members of the PTEN pathway is

sensitive to gene dosage at the 16p11.2 locus. *CHD1L*, finally, was suggested to be a driver of the phenotypes associated with 1q21.1 CNVs [Mefford et al., 2008].

Taken together, these studies described more accurately the clinical phenotypes associated with recurrent deletions or duplications in the 16p11.2 region and pinpointed putative candidate genes. Secondly, the results are in agreement with a 2-locus model for clinically relevant recurrent CNVs, albeit the underlying molecular mechanisms are unexpected and novel [Girirajan and Eichler, 2010]. Thirdly, and most importantly, light is shed on phenotypic effects of perturbed chromosomal organization and on the functioning of the genome as a whole. The phenotypic impact of altered patterns of chromosomal contacts is a novel avenue of research meriting further exploration.

Martin Poot

References

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