When Recessive Genes Mutate to Dominant Gene Action

Examination of pedigrees reveals whether a trait or a disease phenotype is being transmitted in a recessive, dominant or X chromosome-linked mode. For a recessive disease, both alleles of a gene have to be mutated or entirely lost. This may come about if both healthy parents transmit a mutated allele or a loss, or if one transmitted, mutated allele is combined with a second allele that has undergone a de novo mutation or loss. The likelihood of both parents being healthy, heterozygous carriers of mutations depend on the population frequency of mutated alleles, which varies widely among genes. The rate of de novo single nucleotide variation (SNV) is estimated to be approximately $1.0\text{--}1.5 \times 10^{-8}$ and the rate of de novo CNV 1.2×10^{-2} per meiosis [Itsara et al., 2010; Campbell and Eichler, 2013; Rahbari et al., 2016]. These rates depend upon the age of the father and the nucleotide distribution characteristics of the loci in question [Buizer-Voskamp et al., 2013; Francioli et al., 2015; Rahbari et al., 2016]. A few dozens of cases have been reported in which a transmitted or a de novo gene loss ‘unmasks’ a transmitted or a de novo SNV [Flipsen-ten Berg et al., 2007; Poot and Haaf, 2015]. Such ‘unmasking’ has been found more often in patients who exhibit phenotypes reminiscent of 2 syndromes occurring simultaneously than in patients with novel assortments of phenotypes [Hochstenbach et al., 2012; Poot and Haaf, 2015]. Of the latter, a case with 2 overlapping losses affecting a single gene has been re-

ported [Hochstenbach et al., 2012]. Two recent publications challenge this relatively clear-cut picture and may even ‘blur the borders of dominant and recessive inheritance’ [Lemke et al., 2016; Vissing et al., 2016].

In 37 patients of 10 independent pedigrees, a 21-bp in-frame deletion (c.643_663del21) in the *CAPN3* gene cosegregated with signs of muscle disease consistent with autosomal dominant transmission through several generations [Vissing et al., 2016]. The 36 patients who were available for thorough evaluation showed muscle pain, muscle weakness and wasting, fat replacement of muscle tissue, and myopathic changes in muscle biopsies. These phenotypes were generally milder than in patients with autosomal recessive limb girdle muscular dystrophy type 2A but affected the same muscles. The in-frame deletion causes a loss of 7 amino acid residues in the N-terminal calpain-like thiol protease domain of the calpain 3 protein. Western blotting of muscle protein extracts from 9 patients showed less than 15% calpain 3 protein remaining. This level of gene expression cannot be explained by invoking nonsense-mediated mRNA decay. More likely is that the allele with the intragenic deletion suppresses calpain 3 protein concentrations to a very low level, which amounts to dominant negative gene action [Veitia, 2010].

The NMDA receptors are tetrameric ligand-gated ion channels permeable to Na, K, and Ca, which are composed of 2 glycine-binding GluN1 subunits and 2 gluta-

mate-binding GluN2/3 subunits (GluN2A, GluN2B, GluN2C, GluN2D, GluN3A, and GluN3B). GluN1 encoded by *GRIN1* is a ubiquitous component of this receptor. Mutations of the NMDA receptor subunits are associated with a variety of neurodevelopmental phenotypes, including intellectual disability, epilepsy, and autism spectrum disorder. In a meta-analysis of patients with CNVs and SNVs in *GRIN1*, 19 heterozygous de novo mutations clustered within or in direct proximity to the transmembrane domains of the gene [Lemke et al., 2016]. One of the de novo SNVs, p.Ala645Ser, within transmembrane domain 3 and with a Grantham matrix score of 99, was found in a patient with severe intellectual disability, seizures, and cortical visual impairment. This SNV elicited a wild-type response to glutamate, glycine, and Zn in *Xenopus leavis* oocytes analyzed by 2-electrode voltage clamp recording, while all other de novo SNVs elicited impaired or no responses at all. An SNV outside of the transmembrane domains of *GRIN1*, p.Arg217Trp (Grantham matrix score 101), was only pathogenic in the homozygous state. Thus, SNVs in the transmembrane domains exert pathogenic effects if they affect a single *GRIN1* allele, while SNVs elsewhere in the gene are only pathogenic if both *GRIN1* alleles are affected. In addition, heterozygous truncation of *GRIN1* did not result in neurological phenotypes, and deletions encompassing *GRIN1* as well as truncating or splice-site variants were also found in healthy individuals. This suggests that haploinsufficiency of *GRIN1* is tolerated in the healthy human population.

Conceivably, these 2 apparently disparate sets of observations can be subsumed under a single paradigm. The

proteins encoded by the *CAPN3* and *GRIN1* genes have to form multimeric complexes by binding to proteins encoded by other genes. The level of multimer formation would be reduced by those mutations in *CAPN3* and *GRIN1* encoded proteins affecting the domain that binds the other protein. Multimers with such mutant proteins would either be completely inactive, 'leaky', or subject to protein degradation. The CNVs and SNVs affecting the transmembrane domain of *GRIN1* may very well exert a phenotypic effect by producing completely inactive or 'leaky' NMDA receptors [Lemke et al., 2016]. If, on the other hand, the mutant allele of a gene would suppress the expression of the wild-type allele, as was the case in a patient with an intragenic deletion in the *CNTNAP2* gene, significantly less than 50% of wild-type transcript would remain [Lee et al., 2015]. The latter may be the case with the in-frame deletion of *CAPN3* [Vissing et al., 2016]. However plausible these explanations for dominant effects of mutations in transmembrane or dimerization domains of proteins may seem, they need corroboration by functional studies. The cell physiological investigations on *Xenopus leavis* oocytes may be complemented by proteomics and by, for instance, studies of inducible pluripotent stem cells from patients with mutations in these and other genes [Lemke et al., 2016]. The results of such studies may help to broaden our insights into the mechanisms of dominant action of certain mutations in genes that are otherwise known for recessive inheritance. In this way, the present studies not only 'blur the borders of dominant and recessive inheritance', but may also inspire a whole new realm of functional investigation.

Martin Poot

References

- Buizer-Voskamp JE, Blauw HM, Boks MP, van Eijk KR, Veldink JH, et al: Increased paternal age and the influence on burden of genomic copy number variation in the general population. *Hum Genet* 132:443–450 (2013).
- Campbell CD, Eichler EE: Properties and rates of germline mutations in humans. *Trends Genet* 29:575–584 (2013).
- Flipsen-ten Berg K, van Hasselt PM, Eleveld MJ, van der Wijst SE, Hol FA, et al: Unmasking of a hemizygous *WFS1* gene mutation by a chromosome 4p deletion of 8.3 Mb in a patient with Wolf-Hirschhorn syndrome. *Eur J Hum Genet* 15:1132–1138 (2007).
- Francioli LC, Polak PP, Koren A, Menelaou A, Chun S, et al: Genome-wide patterns and properties of de novo mutations in humans. *Nat Genet* 47:822–826 (2015).
- Hochstenbach R, Poot M, Nijman IJ, Renkens I, Duran KJ, et al: Discovery of variants unmasked by hemizygous deletions. *Eur J Hum Genet* 20:748–753 (2012).
- Itsara A, Wu H, Smith JD, Nickerson DA, Romieu I, et al: De novo rates and selection of large copy number variation. *Genome Res* 20:1469–1481 (2010).
- Lee IS, Carvalho CM, Douvaras P, Ho SM, Hartley BJ, et al: Characterization of molecular and cellular phenotypes associated with a heterozygous *CNTNAP2* deletion using patient-derived hiPSC neural cells. *NPJ Schizophr* 1:15019 (2015).
- Lemke JR, Geider K, Helbig KL, Heyne HO, Schütz H, et al: Delineating the *GRIN1* phenotypic spectrum: a distinct genetic NMDA receptor encephalopathy. *Neurology* 86:2171–2178 (2016).
- Poot M, Haaf T: Mechanisms of origin, phenotypic effects and diagnostic implications of complex chromosome rearrangements. *Mol Syndromol* 6:110–134 (2015).
- Rahbari R, Wuster A, Lindsay SJ, Hardwick RJ, Alexandrov LB, et al: Timing, rates and spectra of human germline mutation. *Nat Genet* 48:126–133 (2016).
- Veitia RA: A generalized model of gene dosage and dominant negative effects in macromolecular complexes. *FASEB J* 24:994–1002 (2010).
- Vissing J, Barresi R, Witting N, Van Ghelue M, Gammelgaard L, et al: A heterozygous 21-bp deletion in *CAPN3* causes dominantly inherited limb girdle muscular dystrophy. *Brain* 139:2154–2163 (2016).