A Loss or a Gain, Is It Not All the Same?

The human genome contains numerous loci of which losses or gains provoke clinical phenotypes, albeit often of quite a different nature. Apparently, the genes within these loci have to exist in exactly 2 copies. If there are 3 copies, due to a duplication, or only 1 copy, after a deletion, genes within the locus may exert ‘dosage-sensitive’ effects. This can only be the case if both alleles are being transcribed. If transcripts and proteins of only one copy were to be produced, this would result in haploinsufficiency. Also in the reciprocal situation, in which the cell transcribes 3 copies, transcript imbalance may arise. The gene dosage balance hypothesis provides a molecular framework for how genes encoding transcription factors and proteins involved in signal transduction and macromolecular complexes in general may cause clinical phenotypes [Veitia and Birchler, 2010; Poot et al., 2011].

Disorders resulting from losses or gains of dosage-sensitive genes often show remarkable phenotypic variability. Within the region 7q11.23, a loss causes Williams-Beuren syndrome (WBS; OMIM 194050) and a duplication results in the 7q11.23 duplication syndrome (Dup7; OMIM 609757) [Osborne and Mervis, 2007]. The WBS is characterized by mild to moderate intellectual disability or learning difficulties, with relative cognitive abilities in verbal short-term memory and in language use but weaknesses in visuospatial construction, anxiety, attention deficit hyperactivity disorder, and social disinhibition. Patients with Dup7 syndrome show severely delayed speech and expressive language acquisition but relative good visuospatial functioning. A common inversion within the 7q11.23 region, WBSinv-1, exists as a poly-

morphism in the general population and provokes no phenotype [Tam et al., 2008]. This indicates that severance of the WBS locus within 7q11.23 by a genomic rearrangement would not disrupt a putative transcription-associated domain [Spielmann and Klopocki, 2013; Spielmann and Mundlos, 2013; Poot and Haaf, 2015].

Being flanked by blocks of inverted low copy repeats, the WBS locus within the 7q11.23 region often gives rise to ring(7) chromosomes [Velagaleti et al., 2002]. Patients with a supernumerary ring(7) share speech difficulties, irrespective of whether the ring(7) is present in 100% of the cells or as a high- or low-grade mosaicism [Lichtenbelt et al., 2005]. For a girl with a ring(7) between 12 and 22 Mb in size and present in 50% of lymphocytes, the authors searched for potential candidate genes that may account for her problems with speech acquisition. Bearing in mind that candidate genes should be dosage-sensitive, they pinpointed 2 genes: *STX1A* and *LIMK1*. This reasoning was supported by functional data. The *STX1A* gene-encoded syntaxin protein mediates vesicle docking, fusion, and neurotransmitter release. Overexpression of *STX1A* reduces the nerve growth factor-induced neurite extension in vitro [Zhou et al., 2000]. The *LIMK1* and *LIMK2* genes encode kinases that regulate actin dynamics by phosphorylating cofilin and thus affect neuronal function, neurodevelopment and synaptic plasticity [Cuberos et al., 2015]. Given that this patient carried a 50% mosaicism for ring(7), an increase of only 25% of syntaxin protein and/or LIMK1-encoded kinase activity would be sufficient to account for her clinical phenotypes [Lichtenbelt et al., 2005].

Another patient with a duplication of the WBS locus in 7q11.23 showed moderate to severe language delay at 2 years of age, which worsened to a severe delay in receptive and expressive language at 3 years and 2 months. At 4 years and 2 months, he was diagnosed with attention deficit hyperactivity disorder, overall developmental delay, an unspecified sleep disorder, and a severe delay in speech and language and in fine motor skills [Somerville et al., 2005]. Gene expression analysis in lymphoblastoid cell lines by real-time PCR demonstrated increased expression for 5 of the 6 genes within the duplicated region that were examined (*GTF2I*, *LIMK1*, *WBSCR1*, *RFC2*, and *BAZ1B*). Thus, these data lend support to the previous suggestion of Lichtenbelt et al. [2005] and highlight possible deleterious effects of an even small increase in level of *LIMK1* transcripts.

While most case reports of the WBS and Dup7 syndrome follow the gene-centered paradigm, a recent study took a novel approach to elucidate the effects of reciprocal losses and gains in the genome [Poot and Hochstenbach, 2010; Strong et al., 2015]. They noted that the dup7 locus harbors genes such as *WBSCR22*, which contains an S-adenosyl-L-methionine-binding domain typical of methyltransferases; *NSUN5*, which acts as an RNA-methyltransferase; *BAZ1B*, a subunit of the WICH and B-WICH chromatin remodeling complexes; *BCL7B*, being identified as a mammalian SWI/SNF ATP-dependent subunit of the chromatin-remodeling complex, and the transcrip-

tion factors *GTF2I* and *GTF2IRD1*, which functionally interact with the histone deacetylase HDAC3. This prompted the authors to compare the effects of gene dosage in the region 7q11.23 on genome-wide DNA methylation of whole blood samples from 20 children with WBS, 10 with Dup7 syndrome and 20 samples from age-matched typically developing control children [Strong et al., 2015]. They found gene dose-dependent changes in DNA methylation in genes which were enriched for Gene Ontology categories involved in neurological development, function and disease, albeit that their analysis was done on whole blood-derived DNA. These data suggest that altered DNA methylation and consequential altered gene expression may affect the neurodevelopment in both WBS and Dup7 syndrome. This may, in turn, provoke distinct phenotypes, in particular some of the features of intellectual delay, autism spectrum, speech development, and anxiety disorders. During a decade of study, the WBS/dup(7) locus in the 7q11.23 region has proved a rich source of information regarding developmental disorders, in particular of speech acquisition. In addition, our insights into how changes in DNA copy number may cause clinical phenotypes have progressed from a strictly gene-centered paradigm to a multifaceted approach to the possible modes of gene action. Even 'simple' genome rearrangements such as losses and gains prove now 'complex' in their mechanisms of action and their eventual outcome [Poot and Hochstenbach, 2010; Poot and Haaf, 2015].

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