Double, Double Toil and Trouble

With the complete sequence of the human genome being available, systematic mapping of regions of transcription, transcription factor binding, chromatin structure and histone modification has revealed that 80% of the genome outside of the protein-coding regions performs essential biochemical functions [ENCODE, 2012]. The ‘remainder’ of the human genome contains repeat elements, such as LINEs, SINEs, alpha satellites, and low-copy repeats (LCRs), also known as segmental duplications [Ji et al., 2000; Bailey et al., 2002]. These LCRs are at least 1 kbp in size, show more than 98% homology and predispose the intervening sequences to recombination. Such recombination events produce DNA copy number variations (CNVs), e.g. deletions and duplications, and inversions, which may manifest as genomic disorders [Sharp et al., 2006a]. Such genomic disorders occur with a frequency of 0.7–1.0 per 1,000 live births, often share neurodevelopmental phenotypes and are detected by genome-wide segmental aneuploidy screening [Ji et al., 2000; Hochstenbach et al., 2009, 2011]. CNVs flanked by 2 LCRs are termed recurrent CNVs, since they occur relatively often in cohorts of patients with genomic disorders [Koolen et al., 2006; Sharp et al., 2006b; Mefford et al., 2008; Hannes et al., 2009]. However, since recurrent CNVs also occur in healthy individuals, they themselves are not necessarily pathogenic [Poot et al., 2010]. Indeed, in some patients, 2 recurrent CNVs were found such that concomitant changes in the dosage of genes in both CNVs may account for the clinical phenotype [Girirajan et al.,

2010; Poot et al., 2010]. These findings may explain some of the phenotypic variability among patients with genomic disorders and prompted a 2-hit hypothesis for developmental disorders [Girirajan and Eichler, 2010; Girirajan et al., 2011; Poot et al., 2011].

A special class of rearrangements is located between inverted repeats (IRs), which may render ~12% of the genome susceptible to formation of inversions or of complex duplication-inverted triplication-duplication (DUP-TRP/INV-DUP) rearrangements. Fosmid paired-end sequencing has identified 224 nonredundant inversions in 8 human genomes that were not included in the human reference genome [Kidd et al., 2008]. Recently, IRs were shown to mediate (DUP-TRP/INV-DUP) rearrangements, including the MECP2 duplication syndrome (MIM 300260), Duchenne muscular dystrophy (MIM 310200), VIPR2 triplication, CHRNA7 triplication, and Pelizaeus-Merzbacher disease (MIM 312080) [Carvalho et al., 2011; Shimojima et al., 2012; Beri et al., 2013; Ishmukhametova et al., 2013; Soler-Alfonso et al., 2014]. Duplication of the X-linked proteolipid protein 1 (*PLP1*) gene is the major mutational cause for Pelizaeus-Merzbacher disease and explains ~80% of the cases. This duplication occurs via a mechanism that results in a DUP-TRP/INV-DUP structure [Carvalho et al., 2011; Beck et al., 2015]. An IR distal to *PLP1* facilitates DUP-TRP/INV-DUP formation as well as an inversion, a structural variation found frequently among healthy individuals. *PLP1* duplications were detected in 10 patients with spastic

paraplegia type 2 (MIM 312920), whereas triplications were detected in 6 patients [Beck et al., 2015]. STR markers for these duplicated/triplicated regions were monomorphic in 12 of the 13 patients tested. This indicates that in these patients a single allele was affected by intrachromosomal rearrangement events.

Analysis of the breakpoint junction sequences allows us to identify the underlying mutational process, such as fork stalling and template switching, microhomology-mediated break repair (MMBIR), or a near homologous recombination event between similar Alu elements or LCRs [Hastings et al., 2009]. The nonrecurrent junctions in these patients were consistent with MMBIR [Beck et al., 2015]. This contention is supported by the observation of triplicated and quadruplicated segments, point mutations being associated with some of the breakpoint

junctions, and the presence of intrachromosomal rearrangements [Carvalho et al., 2013; Beck et al., 2015]. Quadruplication and a potentially higher-order amplification of a genomic interval may have resulted from rolling-circle amplification of segments between IRs as predicted by the MMBIR model [Hastings et al., 2009]. The mechanisms for such complex genomic rearrangements have only begun to be elucidated, but the present study has contributed novel insights into this hitherto neglected type of genomic rearrangement [Hastings et al., 2009; Beck et al., 2015]. Although duplications, triplications, quadruplications, etc. do not emerge from the boiling cauldron of the witches in Shakespeare's *Macbeth*, there are more things in our genome than are dreamt of in our philosophy.

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