Genomic Earthquakes in the Human Germline and Their Ramifications

The minimal requirement for a complex chromosome rearrangement (CCR) is that it involves 3 or more chromosome breaks [Madan, 2013; Poot and Haaf, 2015]. This definition was based on decades of examining karyotypes through a microscope. With the advent of molecular cytogenetic techniques, the resolution of the “molecular” karyotype improved, and consequently, the number of detected chromosomal breaks increased. Reexamination of “old” cases revealed that the chromosomal breaks were often accompanied by rearrangements at the breakpoints and also at remote sites in the genome [Kloosterman et al., 2011, 2012; Chiang et al., 2012; Gu et al., 2013; Nazaryan et al., 2014]. Thus, some cases of chromoplexy, translocations involving 3 or more chromosomes, became much more complex [Zhang et al., 2013; Nazaryan and Tommerup, 2016].

During meiosis, chromosomes form pairs. In cases of chromoplexy, this pairing involved formation of complexes with 3, 4, or even more participating chromosomes. Subsequently, these hexavalent, octavalent, and even higher valence chiasmata need to be resolved, such that the translocation chromosomes can separate during the next metaphase. Although the mechanisms of this process are not fully understood, one can assume that the DNA helices need to unwind locally and that double-strand breaks have to form. Conceivably, macromolecular steric hinderance will occur, and the involved DNA molecules will move relative to each other analogous to the rubbing of tectonic plates during an earthquake. Thus, it is likely that at the breakpoints, a small number of nucleotides will be lost. During a subsequent round of DNA replication, this loss will become a small deletion. Alternatively, the lost stretches of DNA may be replicated autonomously and become reintegrated in the chromosome

at the original breakpoint or at a remote site. This has indeed been demonstrated for a case of germline chromothripsis [Slamova et al., 2018]. In this case, joining of the DNA fragments proceeded by canonical NHEJ and alt-NHEJ concomitantly. This suggests that the WRN-encoded 3'→5' exonuclease 3'→5' helicase, which governs preference for either form of NHEJ, may operate on a site-by-site basis.

Taken together, the “molecular signatures” at the breakpoints in cases of chromoplexy closely resemble those remaining after chromothripsis [Poot, 2018]. Distinguishing chromoplexy from chromothripsis may become even more difficult if one considers a hypothetical mechanism proposed by Zhang et al., [2013]. These authors noted that the pairs of adjacent breakpoint ends are less than 1 kbp apart, such that the translocations were almost certainly chained together. If the first chain of 3 translocations occurred all at once, this would have resulted in a functional centromere-containing derivative chromosome. This is retained in the final chromosomal configuration together with 2 mitotically unstable derivatives, which would either be lost or undergo additional rearrangements. The authors suggest that unstable chromosomes generated by a chain of translocations (chromoplexy) could result in subsequent catastrophes adding chromothripsis on top of the original translocations [Zhang et al., 2013]. They termed this model translocation-induced chromothripsis. However plausible such a process based on macromolecular steric hinderance may seem, other, equally plausible, mechanisms of chromothripsis such as micronucleus-based and TREX1 exonuclease-mediated fragmentation of chromosome bridges have been demonstrated [Ly and Cleveland, 2017; Poot, 2018].

A recent study of 83 breakpoint-junctions from 21 cases in which more than one CNV arose simultaneously on a single chromosome indicated that the cell may resort to 3 different types of response [Nazaryan-Petersen et al., 2018]: First, cases with only deletions, but often additional structural rearrangements, such as insertions and inversions, which could be a result of multiple double-strand breaks followed by nonhomologous repair. Second, cases with only duplications or combinations of deletions and duplications, which contained mostly interspersed duplications and breakpoint-junctions enriched with microhomology, which is consistent with serial template switching during DNA replication (chromoanasythesis). Third, rearrangements that were repeat mediated [Nazaryan-Petersen et al., 2016]. Thus, multiple CNVs clustered on a single chromosome may arise through either chromothripsis or chromoanasythesis. The latter resembles the mechanism described for de novo duplications or deletions at the 17p11.2 locus in patients with Potocki-Lupski syndrome or Smith-Magenis syndrome [Beck et al., 2019]. These appear to have resulted from error prone microhomology-mediated break-induced repair (MMBIR) of double-strand breaks. Interestingly, within 1 Mbp from the breakpoints, a significantly elevated level of single nucleotide variants was found. This is reminiscent of kataegis, which accompanies chromothripsis after TREX1 exonuclease-mediated fragmentation of chromosome bridges [Maciejowski et al., 2015].

While cases of chromoplexy or clustered CNVs are rare findings, 2 recent reports describe even more complicated and rarer genome rearrangements [Liu et al., 2017; Hattori et al., 2019]. In a cohort of 60,000 individuals analyzed by genome-wide array CGH, Liu et al. [2017] identified 1,437 individuals with one de novo CNV, 39 with two, 1 with three, 1 with four, and 1 with seven de novo CNVs. These cases are characterized by CCRs, microhomology, and templated insertions at the breakpoints as well as de novo single nucleotide variants in close proximity to breakpoint junctions, which is consistent with a fork stalling and template switching/MMBIR mechanism. The CNVs are predominantly copy number gains (duplications and triplications), which often occurred in tandem. Since all cells in the body were affected, these multiple de novo CNVs (mdnCNVs) must have arisen in a perizygotic interval. Contrary to the “mutator phenotype” in cancer, the driving force behind the mdnCNVs must have been lost after the perizygotic stage, since no further rearrangements leading to somatic mosaicism were detected [Loeb, 2001; Liu et al., 2017]. Thus far, one can only speculate as to the molecular nature of the driv-

ing force behind these mdnCNVs, which most likely arose before the end of meiosis II.

A third case of a highly intricate CCR is clearly distinct from the previously discussed mdnCNVs [Hattori et al., 2019]. Of the 5 affected chromosomes, 2 underwent chromothripsis, a third one an inversion, the fourth and the fifth a tandem duplication, and a deletion with an inverted duplication; each was followed by homologous recombination. The duplicated regions contained both diallelic and triallelic segments, indicating that these genomic rearrangements arose during premeiotic mitosis and were subsequently modified by crossovers during meiosis I. The breakpoints of the rearrangements showed signatures of NHEJ, replication-based errors, and chromothripsis. The rearrangements took place in the paternal germline and were independent of local DNA motifs, which is clearly distinct from the described cases of mdnCNVs in which most rearrangements took place in primary oocytes and postzygotic cells [Liu et al., 2017]. None of the breakpoints in this case were accompanied by de novo single nucleotide variants, as in the previously reported cases of mdnCNVs [Liu et al., 2017]. These characteristics indicate that during spermatogenesis, a transient multifocal genomic crisis can introduce concomitantly multiple chromothriptic and non-chromothriptic rearrangements into the genome. This case broadens the spectrum of mdnCNVs and sets it apart from the “genome chaos” frequently observed in malignant disease [Liu et al., 2014].

Regardless of the multiple, sometimes concomitantly operating, mechanisms provoking such genomic earthquakes, medical genetics is also concerned with the functional consequences of such extremely complicated CCRs. Some 70% of the microscopically detectable CCRs are without phenotypic effect [Pellestor et al., 2011]. Even a germline chromothriptic event, which disrupted 6 protein encoding genes, stably segregated in 11 healthy siblings through 3 generations [Bertelsen et al., 2016]. This family illustrated that heterozygous gene disruptions, involving gene truncation or gene fusion, are not necessarily pathogenic. To decipher the putative phenotypic impact of gene disruptions, the presence of chromatin modifications, as marks for active or silenced genes in the vicinity of the affected genes, have been proposed [Poot and Haaf, 2015]. While this approach may indicate which gene disruptions may be worthwhile of further study, it remains a bioinformatic prediction in need of experimental corroboration.

To resolve a case of Moebius syndrome with a CCR, summarized as 46,XY,t(7;8;11;13), that involved 41 clustered breakpoints, indicating chromothripsis, and trun-

cating 12 protein-encoding genes, a novel approach has been taken [Nazaryan-Petersen et al., 2019]. Starting from the 2 known genes for Moebius syndrome in which mutations have been confirmed (*PLXND1* and *REV3L*), the authors interrogated their 12 disrupted genes in silico for possible interactions with these genes based on experiments, coexpression studies, curated databases, and text mining. Among the 12 truncated protein-coding genes, *SEMA3A* (sema domain, immunoglobulin domain [Ig], short basic domain secreted, semaphorin 3A) is known to bind to *PLXND1* and *PIK3CG* (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit γ) to *REV3L*. This in silico interaction study revealed a functional network encompassing 4 truncated genes, including *SEMA3A*, *SEMA3D* (sema domain, immunoglobulin domain [Ig], short basic domain, secreted, semaphorin 3D), *PIK3CG*, and *UBR5* (ubiquitin protein ligase E3 component n-

recognin 5). Literature on the functions of these genes further supported their possible involvement in Moebius syndrome. Based on the 4 genes making up this functional network, the in silico interaction study further suggested 6 more candidate genes for Moebius syndrome [Nazaryan-Petersen et al., 2019]. A similar approach has recently been taken with regard to genes affected by CNVs in patients with nonsyndromic forms of craniosynostosis [Poot, 2019]. Some of those were found to form a functional network with genes associated with monogenic craniosynostosis syndromes. Searching for functional interactions of genes, being disrupted or lost in sporadic, non-syndromic patients, with genes in syndromic forms of disease may prove helpful for identification of novel candidate genes and for broadening our understanding of the underlying biology of genetic disease in the future.

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