Balanced at First Sight, but in Reality out of Balance

Facing a patient with a complex set of phenotypes, not reminiscent of a Mendelian syndrome, medical geneticists generally decide to perform genome-wide screens for structural genome variations. For decades karyotyping was the first-tier test until it became supplanted by genome-wide arrays [Miller et al., 2010]. Both methods are not mutually exclusive, but rather complement each other. Karyotyping uncovers, albeit with limited resolution, genomic rearrangements, while arrays are limited to detecting copy number variations only. Because of their higher resolution, arrays uncover copy number variations that remained cryptic to karyotyping. With increasing resolution the number of breakpoints discovered also tends to increase [Houge et al., 2003; De Gregori et al., 2007; Sismani et al., 2008; Poot et al., 2009; Kloosterman et al., 2012; Poot and Haaf, 2015]. This experience suggests that many, possibly most, of the thus far detected genome rearrangements may actually be more complex than initially thought.

A case in point was published in a previous issue of *Molecular Syndromology* [Campos et al., 2021]. A boy with developmental delay, in particular regarding language acquisition, microcephaly, failure to thrive, and facial and skeletal dysmorphologies and a G-banded karyotype of 46,XY was reported. His mother's karyotype was 46,XX,t(1; 14; 18; 4) (q? 41; q? 24; q? 21; p? 15). Upon chromosomal microarray he showed two duplications (arr[GRCh37] 1q41q43(219446253–239755240)x3;

4p15.2p14(25803830–37653656)x3). In contrast, his mother showed no imbalances. The authors performed extensive FISH studies to further characterize the rearrangements in the mother which resulted in the following karyotype: 46,XX,ins(1)(pter→p22.3::q41→q43orq43→q41::p22.3→q41::q43→qter),t(4;14;18)(4pter→4p15.2::4p14→4qter;14pter→14q24.1::4p15.2→4p14or4p14→4p15.2::18q21.1→18qter;18pter→18q21.1::14q24.1→14qter). The boy inherited three derivative chromosomes (4, 14, 18), while a fourth abnormal chromosome originated from recombination between the normal and the derivative chromosome 1, resulting in two duplications. His karyotype can be summarized as follows: 46,XY,rec(1)(pter→p22.3::q41→q43orq43→q41::p22.3→qter)ins(1)(p22.3;q41q43)mat, der(14)(14pter→14q24.1::4p15.2→4p14or4p14→4p15.2::18q21.1→18qter)der(18)(18pter→18q21.1::14q24.1→14qter)t(4;14;18)mat. In this final analysis, the boy carried an unbalanced genome rearrangement with at least seven breaks, affecting four chromosomes.

In families with complex chromosome rearrangements clinically affected offspring typically result from transmission of different combinations of derivative and non-rearranged chromosomes. A normal or abnormal phenotype may result, depending on whether the resulting genome is balanced, or the breakpoints disrupt coding or regulatory elements. Since this rearrangement was inherited from the healthy mother, disruption of genes or

topologically associated domains at the breakpoints can be excluded. This patient exhibited a complex clinical picture, involving key features of both the 1q41q43 duplication and the 4p15.2p14 duplication syndromes, which is in agreement with the two duplications detected by chromosomal array.

The discovery of an unbalanced genome, in this case by chromosomal array, should prompt two follow-up investigations. First, it should be ascertained whether the imbalances were de novo or inherited from a healthy parent. Therefore, chromosomal array analyses of both parents are in order. Second, not only the patient but also both parents should be karyotyped to determine what transmitted or de novo rearrangement(s) gave rise to the genomic imbalance. The latter is paramount for determining the recurrence risk of abnormal offspring in the family. The reproductive risks appear to be highly specific for each different kind of rearrangement. A precise estimate may be difficult to give since the population prevalence of complex genome rearrangements is rather low. In this particular family uncovering the balanced translocation in the mother is paramount for future reproductive decisions of both the mother and the patient's sister.

Thus far, meiotic recombination between normal and rearranged homologues resulting in de novo imbalances in the offspring is thought to be very rare. Yet, in recent years several studies of such rearrangements have been published [van Binsbergen et al., 2012; Aristidou et al., 2018; Kurtas et al., 2019]. Translocations involving more than two chromosomes are particularly prone to such additional genome rearrangements [Kloosterman et al., 2012; Nazaryan-Petersen and Tommerup, 2016]. The present case carried an insertion into one of the rearranged chromosomes [Campos et al., 2021]. Such insertion-translocations are difficult to detect, but may underlie some 2.1% of the seemingly de novo, interstitial copy number variations [Nowakowska et al., 2012]. These insertion-translocations differ from tandem duplications in that they often lead to gene disruption and haploinsufficiency [Newman et al., 2015]. The present study is the second one hitherto published [Kurtas et al., 2019; Campos et al., 2021]. This is not to say that such cases are exceptionally rare, for the absence of evidence is no evidence of absence. Only through perseverant and thorough investigations such cases can be uncovered. The present case underscores the need for such investigations in particular in those families in which a seemingly balanced genome rearrangement has been found previously.

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