Genome Medicine

COMMENTARY

Piecing together the problems in diagnosing low-level chromosomal mosaicism

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Abstract

Low-level somatic chromosomal mosaicism, which usually arises from post-zygotic errors, is a known cause of several well defined genetic syndromes and has been implicated in various multifactorial diseases. It is, however, not easy to diagnose, as various physical and technical factors complicate its identification.

Developmental origins of mosaicism

Chromosomal mosaicism is defined as the presence of two or more karyotypically different cell lines in the same individual. Somatic mosaicism mostly results from post-zygotic errors in recombination or replication. Whole chromosomal aneuploidies (in which there are more than or fewer than two copies of one or more chromosomes) can arise by non-disjunction or anaphase lagging. Imbalances of chromosomal segments can originate from unrepaired breakages.

Chromosome instability is common in human cleavage-stage embryos that have been fertilized *in vitro*. This has become more apparent through the application of new techniques for the analysis of the chromosome content of single blastomeres. Recent array comparative genomic hybridization (array CGH) analysis [1] of normally developing, good quality preimplantation embryos confirmed the presence of a high percentage of chromosomal abnormalities at cleavage stage. In addition, this analysis [1] showed that all abnormal embryos were mosaic for the aberrations found and that not only could whole chromosome aneuploidies be detected, but also a significant number of segmental aberrations. Most of these embryos are selected against during the first days and weeks of gestation. As a consequence mosaicism is observed in just 5% of aneuploid spontaneous miscarriages between 6 and 20 weeks [2] and in only 1 to 2% of viable pregnancies screened by chorionic villus sampling [3,4].

The role of somatic mosaicism in disease

Somatic mosaicism contributes to variations in phenotypic expression and disease and has important clinical consequences. Constitutional chromosomal mosaicism has been implicated as a cause of several well described genetic syndromes. The best characterized mosaic syndrome is Pallister-Killian syndrome. This syndrome is a clinically recognizable, multiple malformation syndrome with distinct facial features and is often associated with a diaphragmatic hernia that can lead to neonatal death. It is caused by the presence of an isochromosome 12p, which is an abnormal extra chromosome consisting of two copies of the short arm of chromosome 12 fused at the centromere, resulting in tetrasomy 12p [5,6]. This isochromosome is mainly observed in fibroblasts but is found in only very low numbers in circulating blood lymphocytes [7]. Other such small supernumerary marker chromosomes, defined as structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding techniques alone, are also present in only a subset of cells in the majority of affected individuals [8]. In addition, almost all known chromosomal anomalies have been detected in a mosaic state in occasional patients [9].

The presence of such low-level mosaic chromosome abnormalities has also been linked to several multifactorial diseases. It has, for example, been implicated as a genetic risk factor in children with syndromic autism [10,11]. Moreover, mosaic gains or losses of whole chromosomes have recently been described in the human brain [12,13] and may contribute to certain brain pathologies, such as schizophrenia and Alzheimer's disease [14,15].

Mosaicisms are also important in acquired disorders. Cancer is one of the most prominent forms of somatic mosaicism, and chromosomal copy-number aberrations are a frequent finding in solid tumors [16]. The organism affected with such an alteration is a somatic mosaic, in which the cancerous tissue often has a different genetic constitution from the rest of the body.

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Detection of somatic mosaicism

The true incidence of chromosomal mosaicism causing significant phenotypic effects is unknown but is probably greater than 1 in 10,000. It seems likely, however, that chromosomal mosaicism is underdiagnosed, for two main reasons [17]. First, unless some phenotypic clues (such as discrepancies in body symmetry, pigmentation or facial appearance) indicate the presence of mosaic somatic changes, their occurrence often goes undetected. A study on phenotypically 'normal' individuals using multiplex PCR of seven simple, short tandem repeats showed that trisomies and translocations at each locus examined occurred with an incidence of 1 in 2,000 samples [18,19]. Identification of somatic mosaicism is also clearly hampered by the process of tissue sampling. Aberrations that formed at particular stages of development might be present only in a specific tissue (such as brain tissue or fibroblasts) and could easily be missed in a blood karyotype.

Low-level mosaicisms have long been recognized and were first identified by karyotyping. A common routine test analyzes 20 metaphases from stimulated T-cell cultures, and this enables the detection of an abnormality in 21% or more of the cells with 99% confidence [20]. If the percentage of affected cells is smaller, they can be missed, interpreted as normal variation in the population or dismissed as a culture artifact. Several studies have shown that there is preferential growth of the normal cells in stimulated blood cultures, leading to an underestimation of the percentage of abnormal cells or to the aberration going completely undetected [21-23].

The introduction of fluorescent *in situ* hybridization (FISH) enabled the analysis of large numbers of cells at a higher resolution (about 80 to 200 kilobases) [24] and lower-level mosaicisms could thereby be detected. Unfortunately, FISH looks at only a single specific locus and mosaicism of this locus must therefore be suspected beforehand. With the use of array CGH, genome-wide analysis of mosaic imbalances has become a routine possibility. Several cases of mosaicism have been detected by array CGH [21,22]. Low-level mosaicisms have been described in constitutional disorders, with abnormal cells making up 7 to 30% of cells [22,23,25-27], as well as in cancers [28-30]. The first reports on array CGH showed the ability to detect low-level mosaics, and sporadic reports show that mosaicism as low as 5% can be detected by bacterial artificial chromosome (BAC)-based array CGH [23]. Studies investigating the lower detection limits show that BAC and oligonucleotide arrays enable the detection of 10 to 20% of mosaicisms in a systematic way [21,31]. However, these percentages are reached only if dye swap experiments are performed and no threshold filters are applied, as the deviance from zero might be subtle [31].

The future: improvements and applications

Further increases in sensitivity are warranted for a better diagnosis. First, some smaller copy-number variants can be present in a mosaic form. Second, some aneuploidies can be present in a small minority of the blood cells, the tissue on which tests are most often performed, but can be present in higher numbers in other tissues. For example, the identification of a small percentage of isochromosome 12p in blood cells can enable the diagnosis of Pallister-Killian syndrome.

Finally, methods used in the detection of low grade mosaics may open up new areas of diagnosis in other fields. For example, DNA in the plasma of cancer patients contains circulating DNA derived from the tumor, which might be distinguished from normal DNA by such methods. Equally, it might become possible to detect fetal aneuploidies by screening the plasma of pregnant women. The ability to detect this DNA by genome-wide screening methods would open new diagnostic possibilities.

Abbreviations

Array CGH, array comparative genomic hybridization; FISH, fluorescent *in situ* hybridization.

Authors' contributions

CR and JRV wrote the manuscript. All authors read the manuscript critically.

Competing interests

The authors declare that they have no competing interests.

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