Diagnosis and clinical management of duplications and deletions

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Chromosome deletions and duplications—copy number variations (CNVs)—are a major contribution to the genome variability and can be either pathogenic or not. A particular class, the microdeletions and microduplications, which alter <5 Mb, have been extensively associated with developmental delay and intellectual disability. Although their prevalence in pregnancies and newborn is relatively low, their estimates in preimplantation embryos are poorly defined. The introduction of novel technologies for preimplantation genetic diagnosis of aneuploidies (PGD-A) caused new possibilities and challenges associated with diagnosis of subchromosomal CNVs. Both technical aspects of performing genomewide microarray or next generation sequencing analysis on single cells and interpretation issues are subject of debate. The latter include the reliability of detection of CNVs from embryonic biopsies, their clinical classification based on reproductive outcomes, as well as how before and after test counseling should be organized. It is also important to consider that the current resolution of these technologies from single cells is usually >10 Mb, thus ruling out the possibility to diagnose the most important recurrent microdeletion and microduplication syndromes. Furthermore, at present we face with a lack of well-designed studies addressing the actual resolution and accuracy of CNVs detection in PGD-A and no reference databases is available to evaluate their pathogenicity. Accordingly, it seems reasonable at the moment to avoid the reporting of subchromosomal CNVs in PGD-A. However, although these issues require proper handling, they should not lead us away from providing an improved preimplantation genetic diagnosis. (Fertil Steril® 2017;107:12–8. ©2016 by American Society for Reproductive Medicine.)

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Chromosome abnormalities accounts for approximately 15% of the major congenital anomalies diagnosed before the age of 1 year and are associated with 25% of perinatal deaths (1). Aneuploidies have also been largely associated with infertility and reproductive complications, where they represent the single most important causative factor for implantation failure and miscarriages (2). Fetoplacental aneuploidies account for >50% of sporadic first trimester miscarriages (3), and this rate increases significantly with advancing female age.

In the prenatal setting, chromosome abnormalities have a wide range of genomic imbalances, from polyploidy, to whole chromosome aneuploidy, to submicroscopic deletions and duplications that can only be detected by DNA-based copy number methods, such as fluorescence in situ hybridization or chromosomal microarray (CMA). Of chromosome aneuploidies in spontaneous miscarriages 85%–90% involve whole chromosome copy number alterations (3). Although for these abnormalities the clinical consequences are very well defined and range from embryonic lethality to a few viable autosomal trisomies and sex chromosome copy number aneuploidies, the impact of subchromosomal variations is still a subject of investigation in reproductive genetics. Partial chromosomal deletions and duplications—collectively termed copy number variations (CNVs)—are a major contribution to the genome variability among individuals (4–6) and can be either pathogenic or without clinical consequences. Furthermore, CNVs seems not to be related to female age and originate as an error in the male and female meiosis at apparently similar rates.

The DNA sequence along human chromosomes is constantly changing, and this process enables humans to evolve and adapt (7). About 10 years ago, scientists began to recognize abundant variation of an intermediate size class known as structural variation (8, 9). At present, within this class, CNVs accounts for the largest component. We now typically define the size of CNVs as >50 bp (10), whereas smaller elements are known as insertions or deletions (indels). These structural variations encompass more polymorphic base pairs than SNPs by an order of magnitude (11, 12).

There is a continuous spectrum of phenotypic effects of CNVs, from adaptive traits, to underlining cause of

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disease, to embryonic lethality (13). As technologies have improved to detect smaller and smaller CNVs across the genome, we are learning the very high frequency and important role that this type of genomic variation plays in human diseases. Chromosomal microdeletions and microduplications (MMs) have been associated with syndromic forms of intellectual disability and developmental delay since the 1980s and in the past decade, >200 recurrent MM syndromes have been identified (14).

At present, by evaluating the entire genome at once, previously described syndromes and novel etiologies can be identified faster. The CNV assessment by CMA is becoming recognized as a first-tier test for individuals with intellectual disability and developmental delay in human genetic investigation (15) and represented a paradigm shift in the diagnosis of genetic disorders from ''phenotype-first,'' where clinicians used the patient's phenotype to guide decisions about which genetic tests to consider, to ''genotype-first,'' where clinicians used the patient's genotype to guide their clinical evaluation and management. Given that CNVs are now appreciated as one of the most frequent causes of a broad spectrum of human disorders, early diagnosis and accurate interpretation is important to implement timely interventions and targeted clinical management. The analysis of genomic syndromes using CMA is becoming a common test now also in prenatal diagnosis (PND), especially when amniocentesis or villocentesis is indicated after an abnormal ultrasound result (16). This application became an effective option after the development of large databases incorporating the classification of thousands of normal and pathogenic CNVs that are commonly identified in human pregnancies, although there are still many controversies related to its routine application in the prenatal setting (17, 18). Similar interest has also been recently raised in preimplantation genetics, where the introduction and systematic application of CMA and next-generation sequencing (NGS) technologies hold the potential to improve the detection of subchromosomal abnormalities. The rate limiting step for these high resolution chromosome testing technologies in preimplantation genetics has been, for years, the paucity of starting material that usually consist of a single or few cells collected either at the cleavage or the blastocyst stage. The introduction of whole genome amplification (WGA) protocols has allowed CMAs and NGS protocols to be applied at the embryo biopsy level as micrograms of DNA can be obtained from single cells after the amplification. In the past decade of systematic application of CMA protocols in preimplantation genetic diagnosis for aneuploidies (PGD-A), partial aneuploidies have been reported together with whole chromosome aneuploidies. In this review, a comprehensive evaluation of existing data on the incidence and clinical management of deletions and duplications in the preimplantation setting will be provided in comparison with the prenatal and postnatal setting. There will also be a focus on the strength of evidences for the diagnosis of these subchromosomal abnormalities in embryonic biopsies, as well as a consideration on how these data should be appropriately handled in the clinical setting of an IVF cycle in relation to available scientific evidence.

ORIGIN AND TYPE OF CHROMOSOME DELETIONS AND DUPLICATIONS

There are two major classes of CNVs: recurrent and nonrecurrent. Recurrent CNVs generally arise by nonallelic homologous recombination during meiosis, with breakpoints in the large duplicated blocks of sequence flanking the CNV event. Because the breakpoints cluster within defined regions, the extent of recurrent CNVs is fundamentally identical even in unrelated individuals (19). In contrast, nonrecurrent CNVs have breakpoints that generally lie within unique sequence and do not result from a predisposing genomic architecture. Nonrecurrent CNVs can arise by several mechanisms, including nonhomologous end joining and fork stalling and template switching (20, 21). As a result, although two unrelated individuals may have overlapping nonrecurrent CNVs, they are unlikely to share the same breakpoints. It is possible to estimate that 4.8%–9.5% of the genome contributes to CNV and occasionally it has been reported as up to 100 nondosage sensitive genes can be completely deleted without producing apparent phenotypic consequences.

PREVALENCE OF CHROMOSOME DELETIONS AND DUPLICATION IN THE POSTNATAL, PRENATAL, AND PREIMPLANTATION PERIODS

Postnatal and Prenatal Period

The estimates of prevalence for large chromosomal deletions and duplications are relatively low in the neonatal population. The reported prevalence of chromosomal deletions from congenital anomaly register data ranges from 0.3–2 per 10,000 births (22, 23), with newborn investigation suggesting a similar rate of 0.5–1 per 10,000 (24, 25). A more recent study showed 4.7% of all chromosome abnormalities reported were deletions, including microdeletions, giving a prevalence of 1.99 per 10,000 births (3). Duplications are even less common, showing a prevalence of 0.7 per 10,000 births and representing the 1.6% of all reported chromosome abnormalities (3).

Although large deletions and duplications are rare events, chromosomal MMs make up the most significant fraction of subchromosomal CNVs and a particular class of them have been clearly defined as pathogenic. This special class of recurrent CNVs is termed genomic disorders; mechanistically and phenotypically these are the best-characterized imbalances in the genome (14). Genomic disorder refers to a class of syndromes caused by a partial chromosomal deletion or duplication, usually <5 Mb. spanning several genes that is too small to be detected by conventional cytogenetic methods or high-resolution karyotyping (2–5 Mb). The health and developmental effects associated with MM syndromes can vary tremendously and depend on where in the genome the deletion/duplication is and how many genes it involves. Essentially, these pathogenic CNVs continue to be described in different classes of disease (26). The systematic application of CMAs helped to underline how causative submicroscopic chromosomal imbalances can be found in 10%–15% of patients with DD, multiple congenital abnormalities, or autism,