83e Chromosome Disorders

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CHROMOSOME DISORDERS

Alterations of the chromosomes (numerical and structural) occur in about 1% of the general population, in 8% of stillbirths, and in close to 50% of spontaneously aborted fetuses. The 3×10^9 base pairs that encode the human genome are packaged into 23 pairs of chromosomes, which consist of discrete portions of DNA, bound to several classes of regulatory proteins. Technical advances that led to the ability to analyze human chromosomes immediately translated into the revelation that human disorders can be caused by an abnormality of chromosome number. In 1959, the clinically recognizable disorder, Down syndrome, was demonstrated to result from having three copies of chromosome 21 (trisomy 21). Very soon thereafter, in 1960, a small, structurally abnormal chromosome was recognized in the cells of some patients with chronic myelogenous leukemia (CML), and this abnormal chromosome is now known as the Philadelphia chromosome.

Since these early discoveries, the techniques for analysis of human chromosomes, and DNA in general, have gone through several revolutions, and with each technical advancement, our understanding of the role of chromosomal abnormalities in human disease has expanded. While early studies in the 1950s and 1960s easily identified abnormalities of chromosome number (aneuploidy) and large structural alterations such as deletions (chromosomes with missing regions), duplications (extra copies of chromosome regions), or translocations (where portions of the chromosomes are rearranged), many other types of structural alterations could only be identified as techniques improved. The first important technical advance was the introduction of chromosome banding in the late 1960s, a technique that allowed for the staining of the chromosomes, so that each chromosome could be recognized by its pattern of alternating dark and light (or fluorescent and nonfluorescent) bands. Other technical innovations ranged from the introduction of fluorescence *in situ* hybridization in the 1980s to use of array-based and sequencing technologies in the early 2000s. Currently, we can appreciate that many types of chromosome abnormalities contribute to human disease including aneuploidy; structural alterations such as deletions and duplications, translocations, or inversions; uniparental disomy, where two copies of one chromosome (or a portion of a chromosome) are inherited from one parent; complex alterations such as isochromosomes, markers, and rings; and mosaicism for all of the aforementioned abnormalities. The first chromosome disorders identified had very striking and generally severe phenotypes, because the abnormalities involved large regions of the genome, but as methods have become more sensitive, it is now possible to recognize many more subtle phenotypes, often involving smaller genomic regions.

METHODS FOR CHROMOSOME ANALYSIS

STANDARD CYTOGENETIC ANALYSIS

Standard cytogenetic analysis refers to the examination of banded human chromosomes. Banded chromosome analysis allows for both the determination of the number and identity of chromosomes in the cell and recognition of abnormal banding patterns associated with a structural rearrangement. A stained band is defined as the part of a chromosome that is clearly distinguishable from its adjacent segments by appearing darker or lighter with one or more banding techniques. Cytogenetic analysis is most commonly carried out on cells in mitosis, requiring dividing cells. Actively growing cells are most often obtained from peripheral blood; however, it is only a small subset of the blood cells that are actually used for cytogenetic analysis. Often, chemicals, like phytohemagglutinin (PHA), are used to specially stimulate growth of T cells in a blood sample. Other sources of dividing cells include skin-derived fibroblasts, amniotic fluid or placental tissue (for prenatal diagnosis), or tumor tissue (for cancer diagnosis). After culturing, cells are treated with a mitotic spindle inhibitor, which prevents the separation of the chromatids during metaphase. Halting mitosis in metaphase is essential, because chromosomes are at their most condensed state during this stage of mitosis. The banding pattern of a metaphase chromosome is easily recognizable and is ideal for karyotyping. There are several different types of chromosome staining techniques, including R-banding, C-banding, and quinacrine staining, but the most commonly used is G-banding. G-banding is accomplished by treatment of the chromosomes with a proteolytic enzyme, such as trypsin, which digests some of the proteins holding DNA in a three-dimensional structure, followed by staining with a dye (Giemsa) that binds DNA. The resulting patterns have both dark and light bands; in general, the light bands occur in regions on the chromosome in which genes are actively being transcribed, and dark bands are in regions of less active transcription.

The banded human karyotype has now been standardized based on an internationally agreed upon system for designating not only individual chromosomes but also chromosome regions, providing a way in which structural rearrangements and variants can be described in terms of their composition. The normal human female karyotype is referred to as 46,XX (46 chromosomes, with 22 pairs of autosomes and two of the same type of sex chromosomes [two Xs], indicating this is a female); and the normal human male karyotype is referred to as 46,XY (46 chromosomes, with 22 pairs of autosomes and one of each type of sex chromosome [one X and one Y], indicating this is a male). The anatomy of a chromosome includes the central constriction, known as the centromere, which is critical for movement of the chromosomes during mitosis and meiosis; the two chromosome arms (p for the smaller or petite arm, and q for the longer arm); and the chromosome ends, which contain the telomeres. The telomeres are made up of a hexanucleotide repeat (TTAGGG)n, and unlike the centromere, they are not visible at the light microscope level. Telomeres are functionally important because they confer stability to the end of the chromosome. Broken chromosomes tend to fuse end to end, whereas a normal chromosome with an intact telomere structure is stable. To create the standard chromosome-banding map, each chromosome is divided into segments that are numbered, and then further subdivided. The precise band names are recorded in an international document so that each band has a distinct number. Figure 83e-1 shows an ideogram (chromosome map with bands) of the X chromosome and a G-banded X chromosome. This system provides a way for a chromosome abnormality to be written, with an indication of which band is deleted, duplicated, or rearranged.

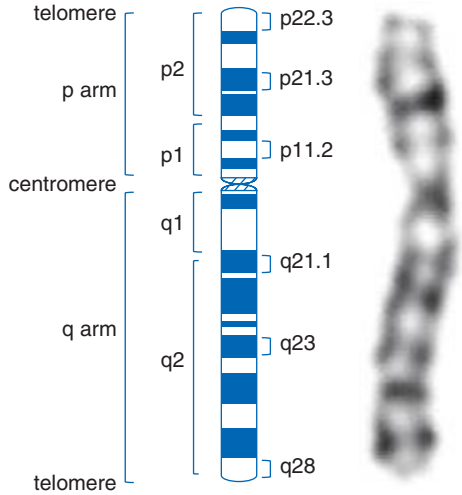

FIGURE 83e-1 Ideogram of the X chromosome and a G-banded X chromosome. The labeling of the X ideogram shows the positioning of the p and q arms, the centromere, and the telomeres. The numbering of the bands is also demonstrated, indicating the broadest subbands (p1, p2, q1, q2) and the further subdivisions to the right. Numbering begins at the centromere and moves out along each arm toward the telomeres.