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# Recent Advances in Cytogenetic Technology for Antenatal Genetic Testing

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The examination of human chromosomes has been a part of the physician's laboratory armamentarium since the correct diploid number of human chromosomes was established[1] and a method was developed[2] for the in vitro growth of peripheral blood leukocytes to yield metaphase chromosomes. The discovery[3] that on ultraviolet microscopy (UV), metaphase chromosomes stained with fluorochrome dyes displayed a characteristic pattern of bright and dull bands unique for a given pair of homologous chromosomes, was a major technological breakthrough in human cytogenetics; for the first time, every chromosome in the karyotype could be unequivocally identified. Although the short storage life of fluorochrome-stained chromosomes and the costs of UV microscopy have limited the usability of fluorescence banding, the introduction of one discriminating procedure quickly led to the development of an array of similar banding techniques for conventional microscopy that yield comparable information. Some of these technical procedures depend on enzyme and/or heat denaturation of the chromosomes, resulting in the characteristic banding patterns seen by the trypsin-Giemsa method,[4] the 5M urea method,[5] and the acid-saline-Giemsa technique.[6] A typical human karyotype prepared from metaphase chromosomes treated with trypsin, stained with Giemsa, and photographed with brightfield photomicrographic techniques is shown in Figure 1. Careful examination of this karyotype reveals that each chromosome in the homologous pair has an array of dark and light bands identical with those of its homolog and that each homologous pair, autosomes number 1 to number 22, has a characteristic, easily identifiable banding pattern.

In order to establish a standardized nomenclature to describe the chromosomes and chromosome regions, as revealed by the banding techniques, a committee of international experts in human cytogenetics met in Paris, France, in 1971.[7] The committee retained the previously established designation of the short arm of the chromosome as "p" and the long arm as "q" and agreed to divide the chromosome arms into a number of regions according to the

This is paper #40 from the Department of Human Genetics of the Medical College of Virginia and was supported in part by a Developmental Disabilities Grant from the Department of Mental Health and Mental Retardation of the Commonwealth of Virginia.

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Fig 1—Normal female karyotype. Chromosomes were treated with trypsin and stained with Giemsa. (Chromosome magnification, ×4000.)

position of the centromere and the banding characteristics of the arm. For example, the long arm of chromosome 7 (7q) (Fig 1) consists of three regions (1 to 3) which are more or less delimited by the two major dark bands and the lighter staining terminal band. If a patient has a deletion in the long arm of this chromosome beginning with the second dark band from the centromere and including all the rest of the long arm to its terminus (ter), the designation would be del (7) (pter → q21:). Such a detailed description of the karyotype abnormality makes it possible to associate the physical abnormalities of a given patient with a given chromosome abnormality and thus establish the phenotype-karyotype correlations which are extremely important in the diagnosis of the so-called chromosome syndromes.

Major indications for a karyotype examination are the evaluation of a newborn with multiple congenital abnormalities, psychomotor retardation of unknown etiology, or ambiguous genitalia; of an adolescent with short stature and/or delayed puberty; of selected cancers such as chronic myelogenous leukemia, retinoblastoma, and cancer of the bladder; of patients with a history of multiple spontaneous abortions or infertility; and of high-risk pregnancies by antenatal genetic testing. Whenever one of the above conditions occurs, aberrations in the chromosomes are a frequent finding. Recently completed newborn surveys show that approximately 1% of all live-born children have a significant chromosome abnormality which leads to severe physical and mental handicap. Chromosome studies of fetal tissues obtained from early spontaneous abortions clearly indicate that karyotype abnormalities are a contributing factor in more than 50% of cases.

The utilization of chromosome banding procedures in antenatal genetic testing has resulted in a more accurate description of the fetal karyotype and increased diagnostic capabilities. Heretofore, it was possible only to document the existence of an abnormal number of chromosomes—a condition known as aneuploidy—as found in Down syndrome, D-trisomy, or E-trisomy, while major or minor structural aberrations went undetected. At the same time, greater expertise in the interpretation of the karyotype result is demanded, and frequently chromosome studies of the parents are essential for a correct interpretation of the cytogenetic findings in the fetus (Figs 2 through 4). The fetal karyotype in Figure 2 shows two chromosomes, chromosome 15 and chromosome 21, with a considerable amount of extra chromatin in the short arm (indicated by arrows), which can easily be construed as an abnormality.

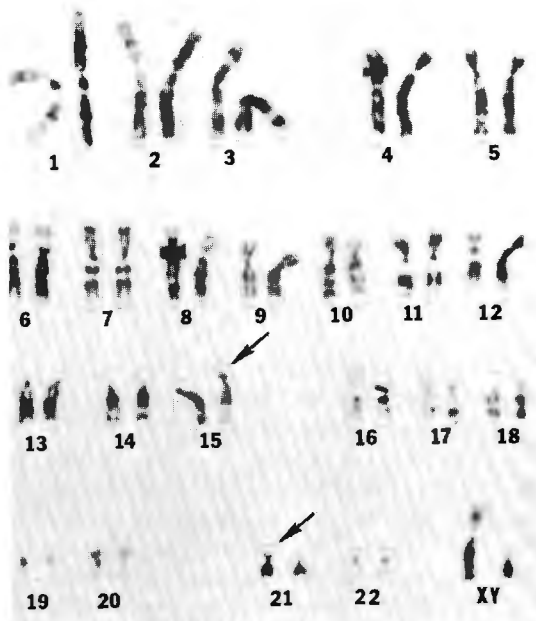


Fig 2—Karyotype of a male fetus. Extra chromatin on the short arm of chromosome 15 and of chromosome 21 is indicated by arrows. (Chromosome magnification, ×4000.)

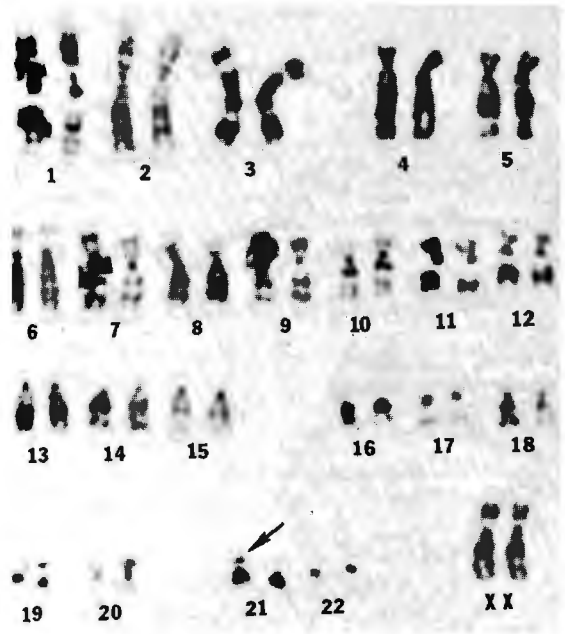


Fig 3—Karyotype of mother showing the polymorphic chromosome 21. (Chromosome magnification, ×4000.)

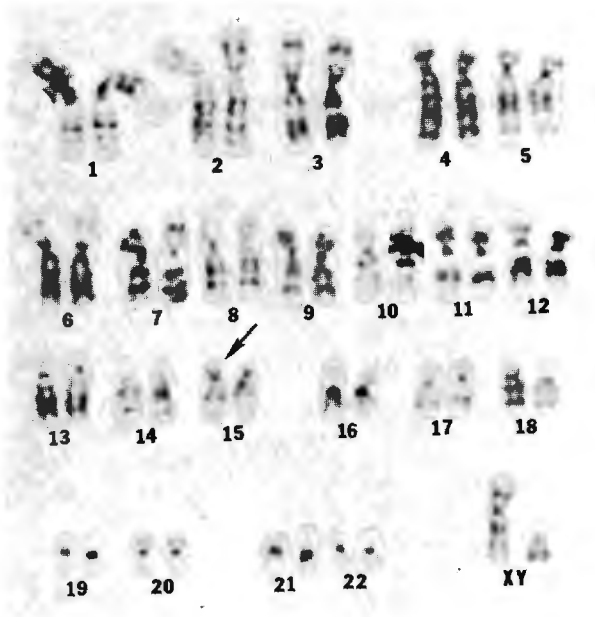


Fig 4—Karyotype of father showing the polymorphic chromosome 15. (Chromosome magnification, ×4000.)

Examination of the parental karyotypes shows that the mother (Fig 3) possesses the variant chromosome 21 and the father (Fig 4) the variant chromosome 15. These chromosomal differences, referred to as normal or polymorphic variants, are often seen in humans with a frequency that depends on the particular chromosome; they are compatible with clinically normal phenotypes. The fetal karyotype in Figure 2, therefore, is normal. Another kind of polymorphism which can confuse the correct interpretation of a fetal karyotype is centromeric heterochromatin (Fig 5). In this case, one of the homologs of chromosome pairs 1, 9, and 16 is larger than its normal homolog—that is, heteromorphic—and the size difference can be attributed to a larger amount of inactive chromatin-heterochromatin as revealed by an alkali treatment of the chromosomes, developed by Arrighi and Hsu.[8] These so-called C-band polymorphisms are encountered in the normal population with a frequency that, depending on the chromosome, shows characteristic racial distributions.

The in vitro culture of amniotic fluid cells for antenatal genetic testing can be complicated in approximately 1% of cases by contamination of the culture with cells of maternal origin. Here, whereas fetal cells can be readily discriminated if the fetus is

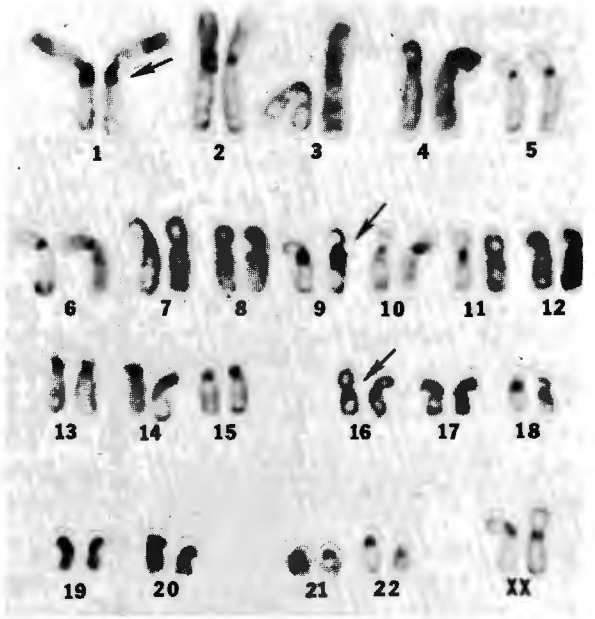


Fig 5—Normal female karyotype. Chromosomes were treated with 0.07N sodium hydroxide and stained with Giemsa. Centromeric heterochromatin is indicated by arrows. (Chromosome magnification, ×4000.)

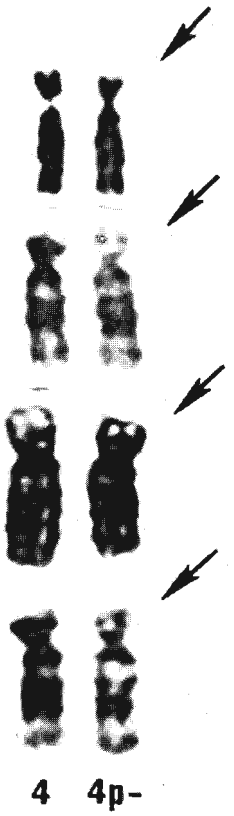


Fig 6—Chromosomes 4 from four different cells of newborn described in text. Arrows indicate the location of the small deletion. (Chromosome magnification, ×4000.)

male, a knowledge and use of chromosome polymorphisms is used when the fetus is female to rule out the presence of maternal cells and assure that the karyotype is fetal. This technical difficulty can further be circumvented by establishing two or more cultures from the amniotic fluid sample and subculturing the cells at least once prior to karyotype evaluation. Assaying duplicate cultures also increases the accuracy of the test where chromosome mosaicism may result in an abnormal offspring.

The application of chromosome banding techniques has vastly increased the precision with which structural rearrangements can be characterized. This in turn has contributed to the mapping of specific genes to specific regions and bands of the chromosome and has led to the recognition of a growing number of syndromes resulting from small duplications, deficiencies, or rearrangements of the chromosome material. Even the smallest duplication or deletion recognizable by current technology can result in major phenotypic abnormalities. For example, Figure 6 shows an array of pairs of chromosome 4 ascertained in a child referred to MCV because of severe multiple congenital anomalies noted at birth. Briefly, this infant female had microcephaly with a midline occipital scalp defect, mild hypotelorism, low-set, simple ears with preauricular pits, bilateral cleft lip and palate, and a bulbous nasal root. She also had mild flexion deformities of both thumbs and hypoplastic nails, as well as congenital heart disease and a shallow pilonidal dimple. Cytogenetic evaluation revealed a modal number of 46 chromosomes, but as this figure demonstrates, one of the chromosomes 4 consistently showed a lesser amount of short arm material than the homolog, and banding analysis revealed a terminal deletion in the short arm: 46,XXdel(4)(p15 or p16).

Unfortunately, in the above case, the cytogenetic diagnosis was made postnatally. Further refinements of our current technology will soon permit such minor chromosome anomalies to be detected prenatally, and as more patients elect antenatal genetic testing and as banding and optical technology are improved, karyotype analysis of the fetus will become an even more precise diagnostic tool.

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