Understanding the Human Karyotype
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SUPPLEMENTAL READING


OBJECTIVES

1. List 2 tissues that would be suitable for chromosome studies in humans.
2. List 6 clinical reasons for doing chromosome studies.
3. Describe the morphological characteristics of human chromosomes.
4. List, using ISCN nomenclature, the chromosomal complements for a normal male and a normal female.
5. List 2 types of chromosome visualization procedures and briefly discuss the uses of each.

I. The Karyotype

*Karyotype* is defined as the use of nomenclature to describe the normal or abnormal, constitutional or acquired, chromosomal complement of an individual, tissue, or cell line.

Why Study Chromosomes?

<table>
<thead>
<tr>
<th>Morbidity/Mortality</th>
<th>Estimate of Cases With Cytogenetic Abnormality</th>
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<tbody>
<tr>
<td>Early embryonic death in unrecognized pregnancies</td>
<td>?? 33%-67%??</td>
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<tr>
<td>Recognized embryonic and fetal deaths (5 weeks or more)</td>
<td>Approximately 30% total; rate varies from 50% at 8-11 weeks to 5% in stillbirths (28 weeks or more gestation)</td>
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<td>Infant and childhood deaths</td>
<td>5% - 7%</td>
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<td>Birth defects</td>
<td>4% - 8%</td>
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<tr>
<td>Congenital heart defects</td>
<td>13%</td>
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<tr>
<td>Sex reversal/ambiguous genitalia</td>
<td>20% - 25%</td>
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<tr>
<td>Multiple miscarriage in couples</td>
<td>2% - 5%</td>
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<td>Females with pubertal anomalies</td>
<td>27%</td>
</tr>
</tbody>
</table>

Characteristics of the Human Karyotype

A. Chromosomes must be obtained from living tissue in which somatic cells are engaged in active cell division (mitosis). Many cells or tissues can be used for karyotype preparation depending on the reason for referral.
Tissues suitable for chromosome studies include:

1. Peripheral blood lymphocytes
2. Bone marrow
3. Chorionic villus biopsy
4. Amniotic fluid cells
5. Fetal blood cells via percutaneous umbilical blood sampling
6. Skin or organ biopsy
B. After obtaining chromosomes from healthy dividing cells, a **karyogram** is usually prepared from photomicrographs or by computer imaging operated by a certified cytogenetic technician.

C. The chromosomes from a cell, individual or species are then arranged (generally by length) in a standard sequence that is referred to as a **karyogram**.

As noted above, knowledge of the human karyotype is important in many areas of clinical medicine. Approximately 3-4% of all births are complicated by a major birth defect and approximately 0.3 - 0.6% of live births are complicated by a chromosome abnormality; also approximately 30%-60% of all first trimester spontaneous pregnancy terminations have an abnormal karyotype.

D. Once the photograph or computer image of the chromosomes from a dividing cell is obtained, the chromosomes are arranged as homologous pairs beginning with the largest pair of autosomes (chromosome pair no. 1) to the smallest pair of autosomes (chromosome pair no. 22). Remember that each homologous pair of chromosomes consists of one maternally and one paternally derived chromosome. These will have been transmitted to the zygote from the egg and sperm at the time of conception. At conception the zygote will then have a constitutional karyotype.
E. The sex chromosomes (XX, for the female and XY, for the male) are usually shown or displayed separately in the karyotype.

F. The normal chromosome number for humans is 46, the diploid number (2n = 46)

The karyotypically normal female is designated: 46,XX.

The karyotypically normal male is designated: 46,XY.

G. The primary morphological characteristics for an individual chromosome include

Centromere

Short arm [p]

Long arm [q]

H. The chromosomes are described according to the shape resulting from the position of the centromere. The shapes seen in human chromosomes include: metacentric, submetacentric, and acrocentric.

**Metacentric** refers to a chromosome in which the centromere is located in the middle of the chromosome (example, chromosome 3).

**Submetacentric** refers to a chromosome in which the centromere is displaced from the center (examples, chromosomes 17 and 18).

**Acrocentric** refers to a chromosome in which the centromere is placed near the end of the chromosome (examples, chromosomes 21 and 22).
II. Chromosome Banding Methods and Procedures Routinely Used in the Clinical Cytogenetics Laboratory

A. History of chromosome banding.

Prior to 1970 it was very difficult to precisely identify specific chromosomes in the human karyotype. While there were some semi-accurate methods, it remained very difficult for cytogeneticists to accurately identify all of the chromosomes in the complement. Thus, it was not possible in many instances to correlate a phenotype with a unique karyotype finding.

In 1970 a Swedish investigator, Dr. Caspersson and colleagues made an astounding discovery which revolutionized the field of clinical cytogenetics. These investigators discovered that if human metaphase chromosomes were stained with either quinacrine HCL (Atebrine) or quinacrine mustard and then viewed with a fluorescence microscope (UV energy source) each homologous pair of human chromosomes had a unique and reproducible series of alternating bright and dull bands along the total linear length of the chromosome. This discovery launched the “banding era”. This discovery ultimately led to our ability to carry out studies on phenotype/karyotype correlations, locate human genes, describe new chromosomal syndromes, correlate a patient’s clinical course with karyotypic findings, and assess the effectiveness of certain chemotherapeutic agents in the treatment of cancer.
III. Ideogram of the Human Karyotype

A. The ideogram of the karyotype is a diagrammatic representation of the karyotype. (See Figure 5.)

B. In humans, the ideogram is generally based on G-banding patterns or staining.

C. The ideogram is arranged and numbered according to the International System for Human Cytogenetic Nomenclature (ISCN, Paris 1971 and U.S.A. 1995).
D. Routine staining method and analysis

Many “chromosome banding” procedures for human metaphase chromosomes have been developed. All of the procedures generally involve some kind of pretreatment and staining specifically tailored to the particular reason for referral.

**G-banding** is the most widely used procedure for routine examination. In general, most patients who are referred for karyotype analysis will have this test performed at the outset. Any additional studies, or “special studies” will be based on what the cytogeneticist observes in these initial G-banded karyotypes. This procedure involves the pretreatment of the chromosomes [with e.g. heat, a proteolytic enzyme (usually
trypsin) or a combination of both] and the subsequent staining of the slide in Giemsa.

IV. Other Staining/Visualization Methods Used in Diagnostic Testing

Particular karyotype findings from routine banding may require the application of additional special procedures in order to facilitate a definitive diagnosis.

A. **High resolution (HR) banding** - by altering the chromosome condensation cycle, very long and extended chromosomes may be obtained with as many as 850+ bands per haploid set. This allows for a very detailed analysis of small aberrations.

![Figure 6](image-url)  
**Figure 6** (Figure from Thompson & Thompson, edition 7, page 63)

B. **Fluorescence in situ hybridization (FISH)** - this is a procedure that provides an opportunity for detailed analysis of missing or extra chromosomal material through the use of a labeled probe (chromosome-specific DNA segment) applied to either interphase nuclei or cells in prophase or metaphase and examined using a fluorescence microscope.  
(Figures 6 - 13 are available in color on the web.)
Figure 7
(Figure from Thompson & Thompson, edition 7, page 63)
C. **SKY** - Spectral Karyotyping is a specialized technology that allows the cytogeneticist to observe **ALL 24** chromosomes simultaneously with chromosome specific dyes. It is extremely helpful in evaluating the chromosomal complement of tumors, or chromosomes with complex rearrangements.

D. **CGH arrays** – Comparative genomic hybridization is a very high resolution technique used to compare two different DNA samples with respect to their relative content of a particular DNA segment or segments. An array is a solid support format that has large numbers of unique DNA fragments.
Figure 11a
(Figure from Thompson & Thompson, edition 7, page 56)

Figure 11b
(Figure from Thompson & Thompson, edition 7, page 64)
Strength of this technique: Allows for the identification of large as well as very small gains or losses in both acquired (cancer) and constitutional cases, thereby leading to identification of changes that are not detectable with standard cytogenetic methods.

Weakness of this technique: Cannot detect balanced rearrangements that are very important in leukemia; cannot detect ploidy changes; can show normal copy number variants that are not of clinical relevance (may require family member studies for interpretation).

V. Reasons for Referring a Patient or a Tissue Sample for Karyotype Analysis.

A. Prenatal

1. Prenatal diagnosis

   a. **Chorionic villus sampling** (CVS) allows the obstetrician to obtain a small sample (~30mg) of tissue (chorion) for subsequent tissue culture and chromosome analysis. The procedure is completed by aspiration biopsy by either transabdominal or transcervical procedures during the first trimester of pregnancy. The results can be obtained in 7 - 14 days and some patients select this procedure because it can be performed so early in the gestation. The risk of complications with this procedure is ~1-3%.

   b. **Amniocentesis** allows the obstetrician to obtain a small sample (~15 - 30 ml.) of amniotic fluid surrounding the developing fetus. The fluid contains viable fetal cells that can be grown in vitro. The sample is usually obtained between 14 and 16 wks. of gestation; the risk of complications is 1/200-1/300 taps.
c. **Percutaneous umbilical blood sampling (PUBS)** is also referred to as **cordocentesis**. Fetal blood is obtained from the umbilical cord and grown in vitro; results are available in a few days. The procedure is done on 2nd-3rd trimester gestations.

d. **All** of the procedures discussed above result in cells that can be used for any number of genetic tests in addition to the chromosome analysis.

2. Pregnancy failure
   a. Stillborn
   b. Multiple spontaneous abortions

B. Postnatal diagnosis

   1. Newborns with congenital malformations
   2. Adolescents with delayed growth and/or sexual development
   3. Adults with a history of infertility
   4. Families with a history of mental retardation
   5. Neoplasms or pre-malignant lesions

![Acute Myelogenous Leukemia (AML)](image)

**Figure 13**

Do all individuals who have a genetic condition (for example, cystic fibrosis) have a chromosomal change?  

**No**

Some genetic changes are too small to be seen on chromosomes.
Practice Problems

What cytogenetic technique would you use to gain information about the chromosomal make-up of the following cases? List the method and give a brief explanation of why you selected this method.

1. A case suspected of having a microdeletion of a specific gene or gene area on chromosome 15 leading to a phenotype suggestive of Prader Willi syndrome.

2. A couple referred because they have a history of 4 spontaneous miscarriages.

3. The acquired chromosomal changes seen in the cells from a patient having lymphoma. In an earlier test, the chromosomal complement of this patient was noted to have multiple chromosomal changes.

4. A child referred because he presents with clinical findings suggestive of sickle cell anemia.
ANSWERS

1. In this case, microdeletions (which by definition are very small) can be difficult to detect with standard GTG-banding. However, if a gene (or gene area) has been identified that is specific for causing the phenotype of a suspected condition (which is true for this case), then one could do a FISH experiment using a locus specific probe.

2. In this case, the best starting study would be to do GTG-banding on peripheral blood samples. Couples having a chromosomal anomaly with a history of spontaneous miscarriages tend to have balanced rearrangements, so the CGH approaches (array or chromosomal) would not be informative. You do not have a target gene to look for, so FISH studies would not be efficient or effective.

3. For this example, you know that there are multiple chromosomal changes present and you have the ability to look at metaphase chromosomes. Since you want to detect all rearrangements, both balanced and unbalanced, GTG-banding could be a strong starting method. However, since you know that there are multiple chromosomal changes SKY would also be a very strong method. It would define each interchromosomal change present.

4. Although sickle cell anemia is a genetic condition, the DNA base pair change that leads to this condition is too small to visualize on a metaphase chromosome. Therefore, since a chromosome test would not provide information about this condition you would not do this assay. Instead, you would request a DNA assay to define the genetic change in the child that resulted in his clinical condition (you will learn more about these types of tests later in this course).