These passages were taken from :Nussbaum RL, McInnes RR, Willard HF. *Thompson & Thompson Genetics in Medicine*. Eighth ed. Philadelphia: Elsevier; 2016.

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Mitosis

During the mitotic phase of the cell cycle, an elaborate apparatus ensures that each of the two daughter cells receives a complete set of genetic information. This result is achieved by a mechanism that distributes one chromatid of each chromosome to each daughter cell (Fig. 2-9). The process of distributing a copy of each chromosome to each daughter cell is called **chromosome segregation** . The importance of this process for normal cell growth is illustrated by the observation that many tumors are invariably characterized by a state of genetic imbalance resulting from mitotic errors in the distribution of chromosomes to daughter cells.

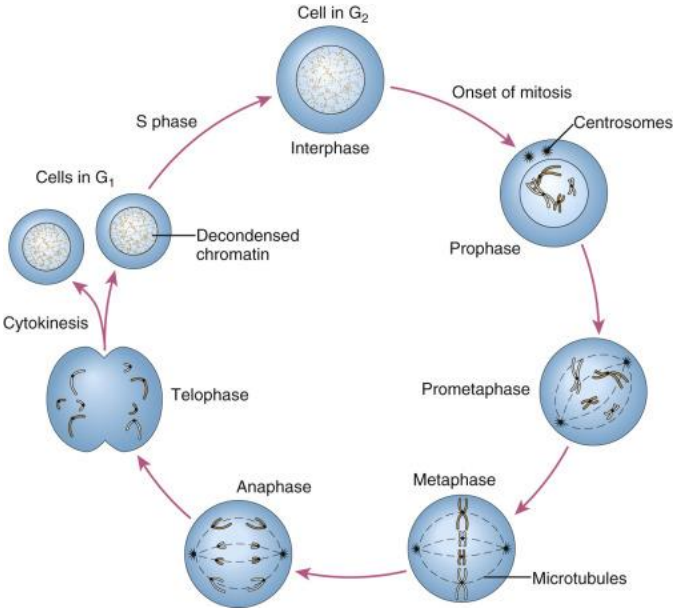


Figure 2-9
Mitosis.
Only two chromosome pairs are shown. For details, see text.

The process of mitosis is continuous, but five stages, illustrated in Figure 2-9, are distinguished: prophase, prometaphase, metaphase, anaphase, and telophase.

Prophase. This stage is marked by gradual condensation of the chromosomes, formation of the mitotic spindle, and formation of a pair of **centrosomes** , from which microtubules radiate and eventually take up positions at the poles of the cell.

Prometaphase. Here, the nuclear membrane dissolves, allowing the chromosomes to disperse within the cell and to attach, by their kinetochores, to microtubules of the mitotic spindle.

Metaphase. At this stage, the chromosomes are maximally condensed and line up at the equatorial plane of the cell.

Anaphase. The chromosomes separate at the centromere, and the sister chromatids of each chromosome now become independent **daughter chromosomes,** which move to opposite poles of the cell.

Telophase. Now, the chromosomes begin to decondense from their highly contracted state, and a nuclear membrane begins to re-form around each of the two daughter nuclei, which resume their interphase appearance. To complete the process of cell division, the cytoplasm cleaves by a process known as **cytokinesis** .

There is an important difference between a cell entering mitosis and one that has just completed the process. A cell in G_2 has a fully replicated genome (i.e., a 4n complement of DNA), and each chromosome consists of a pair of sister chromatids. In contrast, after mitosis, the chromosomes of each daughter cell have only one copy of the genome. This copy will not be duplicated until a daughter cell in its turn reaches the S phase of the next cell cycle (see Fig. 2-8). The entire process of mitosis thus ensures the orderly duplication and distribution of the genome through successive cell divisions.

Meiosis

Meiosis, the process by which diploid cells give rise to haploid gametes, involves a type of cell division that is unique to germ cells. In contrast to mitosis, meiosis consists of one round of DNA replication followed by *two* rounds of chromosome segregation and cell division (see meiosis I and meiosis II in Fig. 2-13). As outlined here and illustrated in Figure 2-14 , the overall sequence of events in male and female meiosis is the same; however, the timing of gametogenesis is very different in the two sexes, as we will describe more fully later in this chapter.

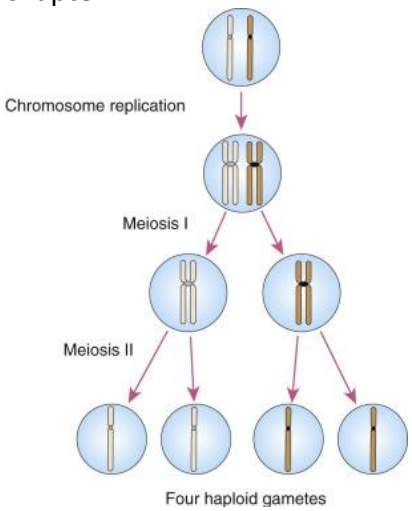


Figure 2-13

A simplified representation of the essential steps in meiosis, consisting of one round of DNA replication followed by two rounds of chromosome segregation, meiosis I and meiosis II.

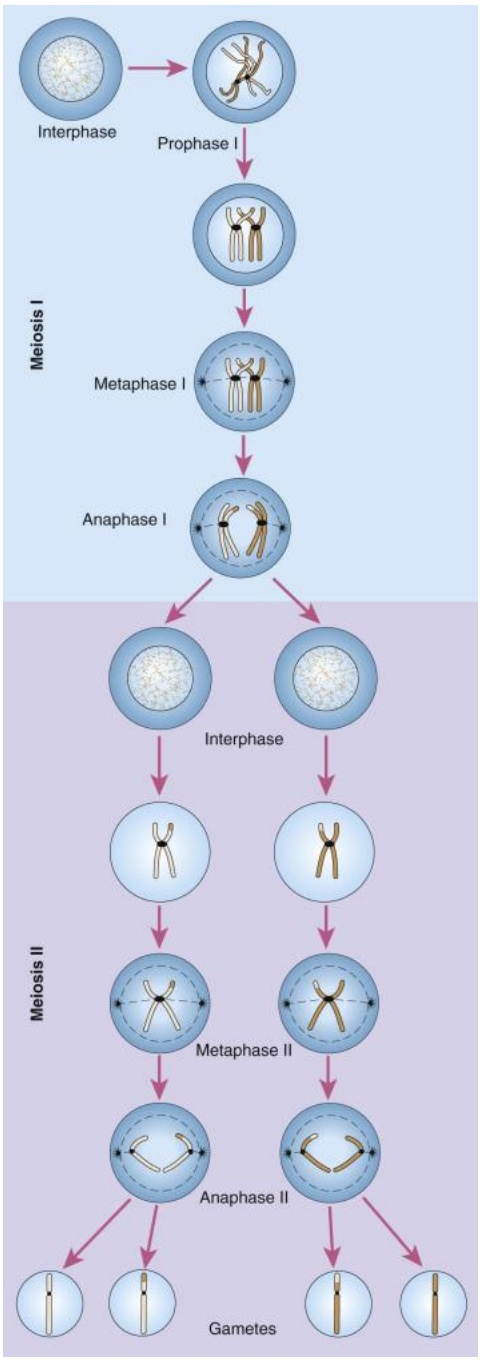


Figure 2-14

Meiosis and its consequences.

A single chromosome pair and a single crossover are shown, leading to formation of four distinct gametes. The chromosomes replicate during interphase and begin to condense as the cell enters prophase of meiosis I. In meiosis I, the chromosomes synapse and recombine. A crossover is visible as the homologues align at metaphase I, with the centromeres oriented toward opposite poles. In anaphase I, the exchange of DNA between the homologues is apparent as the chromosomes are pulled to opposite poles. After completion of meiosis I and

cytokinesis, meiosis II proceeds with a mitosis-like division. The sister kinetochores separate and move to opposite poles in anaphase II, yielding four haploid products.

Meiosis I is also known as the **reduction division** because it is the division in which the chromosome number is reduced by half through the pairing of homologues in prophase and by their segregation to different cells at anaphase of meiosis I. Meiosis I is also notable because it is the stage at which genetic **recombination** (also called **meiotic crossing over**) occurs. In this process, as shown for one pair of chromosomes in Figure 2-14 , homologous segments of DNA are exchanged between nonsister chromatids of each pair of homologous chromosomes, thus ensuring that none of the gametes produced by meiosis will be identical to another.

Prophase of meiosis I differs in a number of ways from mitotic prophase, with important genetic consequences, because homologous chromosomes need to pair and exchange genetic information. The most critical early stage is called **zygotene,** when homologous chromosomes begin to align along their entire length. The process of meiotic pairing—called **synapsis** —is normally precise, bringing corresponding DNA sequences into alignment along the length of the entire chromosome pair. The paired homologues—now called **bivalents** —are held together by a ribbon-like proteinaceous structure called the **synaptonemal complex,** which is essential to the process of recombination. After synapsis is complete, meiotic crossing over takes place during **pachytene,** after which the synaptonemal complex breaks down.

Metaphase I begins, as in mitosis, when the nuclear membrane disappears. A spindle forms, and the paired chromosomes align themselves on the equatorial plane with their centromeres oriented toward different poles (see Fig. 2-14).

Anaphase of meiosis I again differs substantially from the corresponding stage of mitosis. Here, it is the two members of each bivalent that move apart, not the sister chromatids (contrast Fig. 2-14 with Fig. 2-9). The homologous centromeres (with their attached sister chromatids) are drawn to opposite poles of the cell, a process termed **disjunction** . Thus the chromosome number is halved, and each cellular product of meiosis I has the haploid chromosome number. The 23 pairs of homologous chromosomes assort independently of one another, and as a result, the original paternal and maternal chromosome sets are sorted into random combinations. The possible number of combinations of the 23 chromosome pairs that can be present in the gametes is 2^{23} (more than 8 million). Owing to the process of crossing over, however, the variation in the genetic material that is transmitted from parent to child is actually much greater than this. As a result, each chromatid typically contains segments derived from each member of the original parental chromosome pair, as illustrated schematically in Figure 2-14. For example, at this stage, a typical large human chromosome would be composed of three to five segments, alternately paternal and maternal in origin, as inferred from DNA sequence variants that distinguish the respective parental genomes (Fig. 2-15).

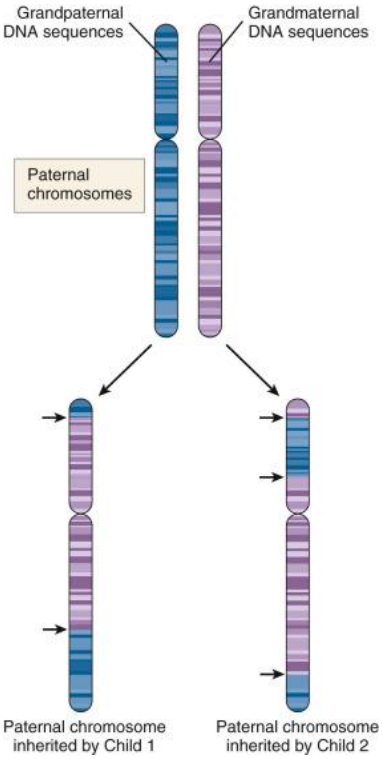


Figure 2-15

The effect of homologous recombination in meiosis.

In this example, representing the inheritance of sequences on a typical large chromosome, an individual has distinctive homologues, one containing sequences inherited from his father (*blue*) and one containing homologous sequences from his mother (*purple*). After meiosis in spermatogenesis, he transmits a single complete copy of that chromosome to his two offspring. However, as a result of crossing over (*arrows*), the copy he transmits to each child consists of alternating segments of the two grandparental sequences. Child 1 inherits a copy after two crossovers, whereas child 2 inherits a copy with three crossovers.

After telophase of meiosis I, the two haploid daughter cells enter meiotic interphase. In contrast to mitosis, this interphase is brief, and meiosis II begins. The notable point that distinguishes meiotic and mitotic interphase is that there is no S phase (i.e., no DNA synthesis and duplication of the genome) between the first and second meiotic divisions.

Meiosis II is similar to an ordinary mitosis, except that the chromosome number is 23 instead of 46; the chromatids of each of the 23 chromosomes separate, and one chromatid of each chromosome passes to each daughter cell (see Fig. 2-14). However, as mentioned earlier, because of crossing over in meiosis I, the chromosomes of the resulting gametes are not identical (see Fig. 2-15).

Nondisjunction

Although the causes of aneuploidy are not fully understood, the most common chromosomal mechanism is **meiotic nondisjunction** . This refers to the failure of a pair of chromosomes to disjoin properly during one of the two meiotic divisions, usually during meiosis I. The genomic consequences of nondisjunction during meiosis I and meiosis II are different (Fig. 5-10). If the

error occurs during meiosis I, the gamete with 24 chromosomes contains both the paternal and the maternal members of the pair. If it occurs during meiosis II, the gamete with the extra chromosome contains both copies of either the paternal or the maternal chromosome. (Strictly speaking, these statements refer only to the paternal or maternal centromere, because recombination between homologous chromosomes has usually taken place in the preceding meiosis I, resulting in some genetic differences between the chromatids and thus between the corresponding daughter chromosomes; see Chapter 2 .)

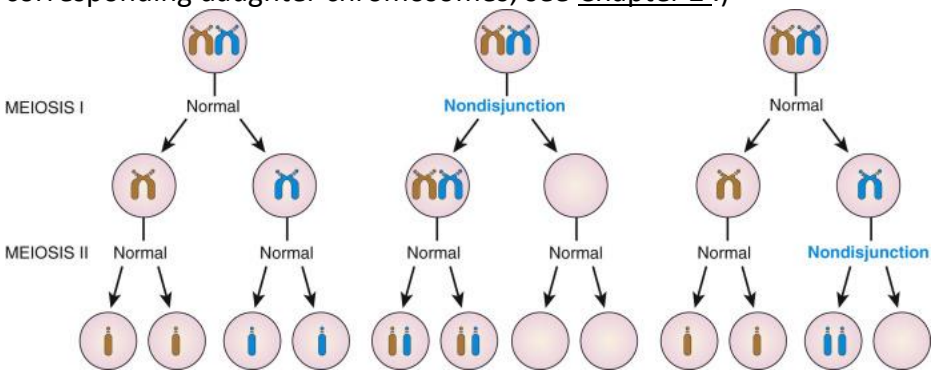


Figure 5-10
The different consequences of nondisjunction at meiosis I (*center*) and meiosis II (*right*) , compared with normal disjunction (*left*). If the error occurs at meiosis I, the gametes either contain a representative of both members of the chromosome 21 pair or lack a chromosome 21 altogether. If nondisjunction occurs at meiosis II, the abnormal gametes contain two copies of one parental chromosome 21 (and no copy of the other) or lack a chromosome 21.

Proper disjunction of a pair of homologous chromosomes in meiosis I appears relatively straightforward (see Fig. 5-10). In reality, however, it involves a feat of complex engineering that requires precise temporal and spatial control over alignment of the two homologues, their tight connections to each other (synapsis), their interactions with the meiotic spindle, and, finally, their release and subsequent movement to opposite poles and to different daughter cells. The propensity of a chromosome pair to nondisjoin has been strongly associated with aberrations in the frequency or placement, or both, of recombination events in meiosis I, which are critical for maintaining proper synapsis. A chromosome pair with too few (or even no) recombinations, or with recombination too close to the centromere or telomere, may be more susceptible to nondisjunction than a chromosome pair with a more typical number and distribution of recombination events.
In some cases, aneuploidy can also result from premature separation of sister chromatids in meiosis I instead of meiosis II. If this happens, the separated chromatids may by chance segregate to the oocyte or to the polar body, leading to an unbalanced gamete.
Nondisjunction can also occur in a mitotic division after formation of the zygote. If this happens at an early cleavage division, clinically significant **mosaicism** may result (see later section). In some malignant cell lines and some cell cultures, mitotic nondisjunction can lead to highly abnormal karyotypes.

Trinucleotide-Repeat Disorders: Unstable Mutations

THE FOLLOWING is taken from: Gilner JB, Kuller JA, Valea FA. Reproductive genetics. In: Lobo RA, Gershenson DM, Lentz GM, Valea FA, eds. Comprehensive Gynecology. 7th ed. Philadelphia, PA: Elsevier; 2017.https://app.medicine.wsu.edu/proxima?url=https://www.clinicalkey.com/#!/content/book/3-s2.0-B9780323322874000028?scrollTo=%23hl0000985

In the early 1990s a new class of genetic conditions was recognized as being due to unstable dynamic mutations in a gene. In classic genetic inheritance, the diseases and their inheritance patterns are due to mutations that are passed on from generation to generation in a stable form. That is, all affected members in a family have the identical inherited mutation. In 1991, however, a number of reports began to describe a new class of genetic condition in which the gene mutation was dynamic and would change with different affected individuals within a family. The most common group of disorders is known as *triplet* , or *trinucleotide repeat, disorders* . More than a dozen diseases are now known to be associated with unstable trinucleotide repeats (Table 2.3) (Cummings, 2000).

Table 2.3

Some Commonly Known Disorders Associated with Unstable Triplet Repeats

				Repeat Number		
Disease	**Inheritance Pattern**	**Triplet Repeat**	**Location of Expansion**	**Normal**	**Unstable**	**Affected**
Huntington disease	Autosomal dominant	CAG	Exon coding region	<36	29-35	>35
Fragile X	X-linked	CGG	5′ untranslated region	<55	56-200	>200
Myotonic dystrophy	Autosomal dominant	GTG	3′ untranslated region	<35	50-100	>100
Spinal cerebellar ataxias *	Autosomal dominant	CAG	Exon	<40	Different for each subtype	>40
Friedrich ataxia	Autosomal recessive	GAA	Intron of gene	<33	34-65	>65

View full size

∗ Spinal cerebellar ataxias are a heterogeneous group of conditions, all of which appear to be associated with a CAG repeat. Each subtype has its own specific range of normal, unstable, and affected repeat sizes.

These conditions are characterized by an expansion of variable size, within the affected gene, of a segment of DNA that contains a repeat of three nucleotides such as CAGCAGCAG (CAG) *n* , or CCGCCG (CCG) *n* . These triplet repeats are unstable in that they tend to expand as the gene is passed on from generation to generation. The molecular mechanism is most likely misalignment at the time of meiosis. The result of increasing triplet expansion is progressively earlier onset or more severe manifestation of disease with each successive generation. This phenomenon is known as *anticipation* .

The commonality of this group of genetic conditions stops at the shared molecular mechanism. Each disease, otherwise, has its own features. Some, such as myotonic dystrophy, are inherited in an autosomal dominant pattern, but others, such as Friedrich ataxia, are autosomal recessive conditions. The susceptibility of the triplet repeat to expand also may depend on the parent of origin: paternal in Huntington disease and exclusively maternal in fragile X syndrome.

Fragile X Syndrome

Fragile X syndrome, a disease within the unstable triplet group, is the most common heritable form of moderate mental retardation and is second to Down syndrome among the causes of mental retardation in males. In women, a mild carrier state may present as premature menopause. The gene is located on the X chromosome at Xq27.3 and causes a pattern of abnormalities, including mental retardation and characteristic facial features. Disease frequency is approximately 1 in 4000 male births. The condition is due to an expansion of the triplet repeat CGG located in the untranslated region of the first exon of the gene called *FMR1* (fragile X mental retardation 1). The triplet expansion blocks normal function of the *FMR1* gene, thus causing the syndrome.

Normal individuals have about 8 to 50 copies of the CGG triplet, whereas affected individuals have from 200 to more than 1000 copies. Individuals with an intermediate number of copies (52 to 200) are known as *premutation carriers* ; this level of “expansion” renders the triplet-repeat segment unstable. These carriers are generally unaffected but are at risk for having affected children or descendants if the premutation expands in successive generations. The premutation, however, can be passed on without expanding.

Long-term follow-up of premutation carriers has revealed that these individuals are not necessarily “unaffected.” Premature ovarian failure has been associated with female premutation carriers, and in men, a syndrome of atypical adult-onset ataxia (FXTAS) has now been described (Hagerman, 2004).

Although the unstable triplet is transmitted in an X-linked pattern, the probabilities of the different phenotypes are far from traditional X-linked inheritance. Understanding of this feature of the fragile X syndrome is crucial to genetic counseling and assessing recurrence risks. The possible outcomes of the offspring of a premutation carrier female are the following:

- 1. Male offspring—three possibilities:
    - a. Unaffected by not having inherited the X chromosome with the premutation.
    - b. Unaffected by inheriting the X chromosome with the premutation, which did *not* expand (about 20% of the time); this male, however, is at risk for passing the premutation to his daughters, who in turn will be at risk for having affected children. Therefore, for this male, his grandchildren will be at risk for the fragile X syndrome.

    - c. Affected by having inherited the abnormal X chromosome, in which the premutation also expanded to a full mutation.
- 2. Female offspring—four possibilities:
    - a. Unaffected by not having inherited the X chromosome with the premutation.
    - b. Unaffected by inheriting the X chromosome with the premutation that did *not* expand.
    - c. Unaffected, but inherited the X chromosome with an expansion—about 50% of females with the expansion appear to be clinically unaffected.
    - d. Affected by inheriting the X chromosome with an expansion.

THE FOLLOWING is taken from:
Kumar V, Abbas AK, Aster JC. Genetic disorders. In: Kumar V, Abbas AK, Aster JC, eds. Robbins and Cotran Pathologic Basis of Disease. 9th ed. Philadelphia, PA: Elsevier; 2015. https://www.clinicalkey.com/#!/content/book/3-s2.0-B9781455726134000050.

Genomic Imprinting

We all inherit two copies of each autosomal gene, carried on homologous maternal and paternal chromosomes. In the past, it had been assumed that there is no functional difference between the alleles derived from the mother or the father. Studies over the past two decades have provided definite evidence that, at least with respect to some genes, important functional differences exist between the paternal allele and the maternal allele. These differences result from an epigenetic process called *imprinting.* In most cases, imprinting selectively inactivates either the maternal or paternal allele. Thus, *maternal imprinting* refers to transcriptional silencing of the maternal allele, whereas *paternal imprinting* implies that the paternal allele is inactivated.

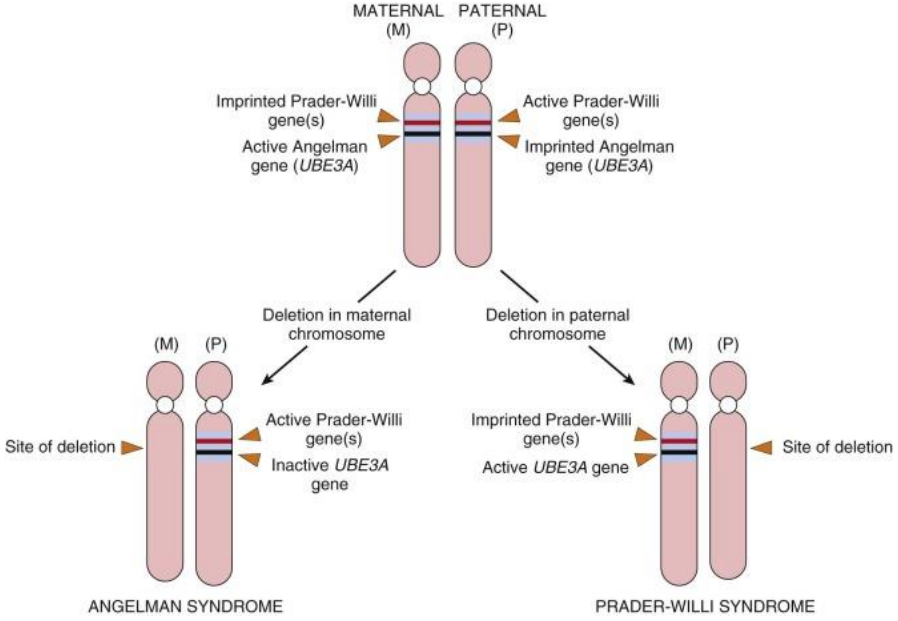
Imprinting occurs in the ovum or the sperm, before fertilization, and then is stably transmitted to all somatic cells through mitosis. As with other instances of epigenetic regulation, imprinting is associated with differential patterns of DNA methylation at CG nucleotides. Other mechanisms include histone H4 deacetylation and methylation. Regardless of the mechanism, it is believed that such marking of paternal and maternal chromosomes occurs during gametogenesis, and thus it seems that from the moment of conception some chromosomes remember where they came from. The exact number of imprinted genes is not known; estimates range from 200 to 600. Although imprinted genes may occur in isolation, more commonly they are found in groups that are regulated by common *cis* -acting elements called imprinting control regions. Genomic imprinting is best illustrated by considering two uncommon genetic disorders: Prader-Willi syndrome and Angelman syndrome which were originally believed to be unrelated until the genetic lesions responsible for them were mapped to the very same location. They are described next.

Prader-Willi Syndrome and Angelman Syndrome

Prader-Willi syndrome is characterized by mental retardation, short stature, hypotonia, profound hyperphagia, obesity, small hands and feet, and hypogonadism. In 65% to 70% of cases, an interstitial deletion of band q12 in the long arm of chromosome 15, del(15)(q11.2q13), can be detected. In most cases the breakpoints are the same, causing a 5-Mb deletion. *It is striking that in all cases the deletion affects the paternally derived chromosome 15.* In contrast with the Prader-Willi syndrome, patients with the phenotypically distinct Angelman syndrome are *born with a deletion of the same chromosomal region derived from their mothers.* **Patients with Angelman syndrome are also mentally retarded, but in addition they present with ataxic gait, seizures, and inappropriate laughter.** Because of their laughter and ataxia, they have been referred to as “happy puppets.” A comparison of these two syndromes clearly demonstrates the *parent-of-origin* effects on gene function.

The molecular basis of these two syndromes lies in the genomic imprinting (Fig. 5-28). Three mechanisms are involved:

- *Deletions* . It is known that a gene or set of genes on maternal chromosome 15q12 is imprinted (and hence silenced), and thus the only functional allele(s) are provided by the paternal chromosome. When these are lost as a result of a deletion, the person develops Prader-Willi syndrome. Conversely, a distinct gene that also maps to the same region of chromosome 15 is imprinted on the paternal chromosome. Only the maternally derived allele of this gene is normally active. Deletion of this maternal gene on chromosome 15 gives rise to the Angelman syndrome. Deletions account for about 70% cases.
- *Uniparental disomy.* Molecular studies of cytogenetically normal patients with the Prader-Willi syndrome (i.e., those without the deletion) have revealed that they have two maternal copies of chromosome 15. Inheritance of both chromosomes of a pair from one parent is called *uniparental disomy.* The net effect is the same (i.e., the person does not have a functional set of genes from the [nonimprinted] paternal chromosomes 15). Angelman syndrome, as might be expected, can also result from uniparental disomy of paternal chromosome 15. This is the second most common mechanism responsible for 20% to 25% cases.
- *Defective imprinting.* In a small minority of patients (1% to 4%), there is an imprinting defect. In some patients with Prader-Willi syndrome, the paternal chromosome carries the maternal imprint and conversely in Angelman syndrome the maternal chromosome carries the paternal imprint (hence there are no functional alleles).



Diagrammatic representation of Prader-Willi and Angelman syndromes.

The genetic basis of these two imprinting disorders is now being unraveled.

- In the Angelman syndrome, the affected gene is a ubiquitin ligase that is involved in catalyzing the transfer of activated ubiquitin to target protein substrates. The gene, called *UBE3A,* maps within the 15q12 region, is imprinted on the paternal chromosome, and is expressed from the maternal allele primarily in specific regions of the brain. The imprinting is tissue-specific in that *UBE3A* is expressed from both alleles in most tissues.

- In contrast to Angelman syndrome, no single gene has been implicated in Prader-Willi syndrome. Instead, a series of genes located in the 15q11.2-q13 interval (which are imprinted on the maternal chromosome and expressed from the paternal chromosome) are believed to be involved. These include the SNORP family of genes that encode small nucleolar RNAs which are involved in modifications of ribosomal RNAs. Loss of SNORP functions is believed to contribute to Prader-Willi syndrome.

Molecular diagnosis of these syndromes is based on assessment of methylation status of marker genes and FISH. The importance of imprinting is not restricted to rare chromosomal disorders. Parent-of-origin effects have been identified in a variety of inherited diseases, such as Huntington disease and myotonic dystrophy and in tumorigenesis.

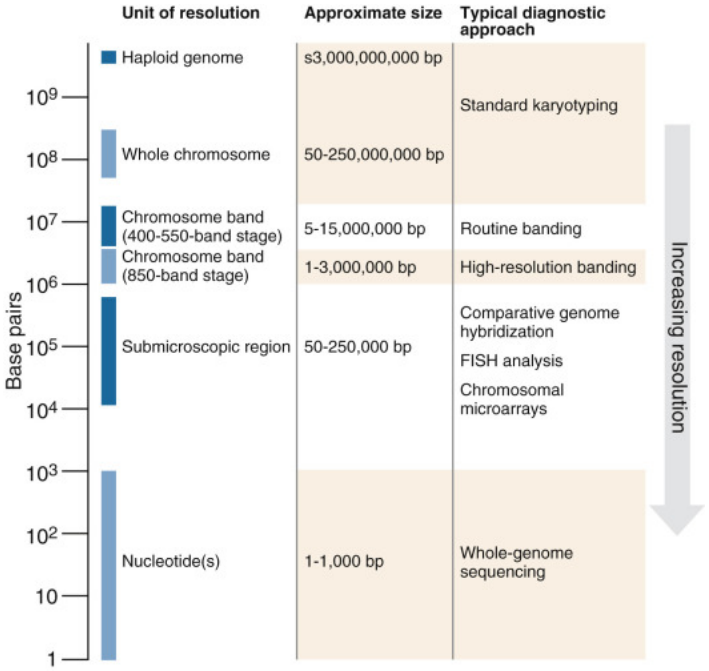
KEY CONCEPTS

Genomic Imprinting

- Imprinting involves transcriptional silencing of the paternal or maternal copies of certain genes during gametogenesis. For such genes, only one functional copy exists in the individual. Loss of the functional (not imprinted) allele by deletion gives rise to diseases.
- In **Prader- Willi syndrome,** deletion of band q12 on long arm of paternal chromosome 15 occurs. Genes in this region of maternal chromosome 15 are imprinted so there is complete loss of their functions. Patients have mental retardation, short stature, hypotonia, hyperphagia, small hands and feet, and hypogonadism.
- In **Angelman syndrome** there is deletion of the same region from the maternal chromosome. Since genes on the corresponding region of paternal chromosome 15 are imprinted, these patients have mental retardation, ataxia, seizures, and inappropriate laughter.

THE FOLLOWING is taken from: Nussbaum RL, McInnes RR, Willard HF. Principles of clinical cytogenetics and genome analysis. In : Nussbaum RL, McInnes RR, Willard HF, eds. Thompson & Thompson Genetics in Medicine. 8th ed. Philadelphia, PA: Elsevier; 2016. https://www.clinicalkey.com/#!/content/book/3-s2.0-B9781437706963000054.

Clinical cytogenetics is the study of chromosomes, their structure, and their inheritance, as applied to the practice of medicine. It has been apparent for over 50 years that chromosome abnormalities—microscopically visible changes in the number or structure of chromosomes—could account for a number of clinical conditions that are thus referred to as **chromosome disorders** . With their focus on the complete set of genetic material, cytogeneticists were the first to bring a genome-wide perspective to the practice of medicine. Today, chromosome analysis—with increasing resolution and precision at both the cytological and genomic levels—is an important diagnostic procedure in numerous areas of clinical medicine. Current genome analyses that use approaches to be explored in this chapter, including **chromosomal microarrays** and **whole-genome sequencing,** represent impressive improvements in capacity and resolution, but ones that are conceptually similar to microscopic methods focusing on chromosomes.



Spectrum of resolution in chromosome and genome analysis.
The typical resolution and range of effectiveness are given for various diagnostic approaches used routinely in chromosome and genome analysis. See text for details and specific examples. FISH, Fluorescence in situ hybridization.

Chromosome disorders form a major category of genetic disease. They account for a large proportion of all reproductive wastage, congenital malformations, and intellectual disability and play an important role in the pathogenesis of cancer. Specific cytogenetic disorders are responsible for hundreds of distinct syndromes that collectively are more common than all the single-gene diseases together. Cytogenetic abnormalities are present in nearly 1% of live births,

in approximately 2% of pregnancies in women older than 35 years who undergo prenatal diagnosis, and in fully half of all spontaneous, first-trimester abortions.
The spectrum of analysis from microscopically visible changes in chromosome number and structure to anomalies of genome structure and sequence detectable at the level of whole-genome sequencing encompasses literally the entire field of medical genetics (see the figure above). In this chapter, we present the general principles of chromosome and genome analysis and focus on the **chromosome mutations** and **regional mutations** introduced in the previous chapter.

Introduction to Cytogenetics and Genome Analysis

To be examined by chromosome analysis for clinical purposes, cells must be capable of proliferation in culture. The most accessible cells that meet this requirement are white blood cells, specifically T lymphocytes. To prepare a short-term culture that is suitable for cytogenetic analysis of these cells, a sample of peripheral blood is obtained, and the white blood cells are collected, placed in tissue culture medium, and stimulated to divide. After a few days, the dividing cells are arrested in **metaphase** with chemicals that inhibit the mitotic spindle. Cells are treated with a hypotonic solution to release the chromosomes, which are then fixed, spread on slides, and stained by one of several techniques, depending on the particular diagnostic procedure being performed. They are then ready for analysis.

Although ideal for rapid clinical analysis, cell cultures prepared from peripheral blood have the disadvantage of being short-lived (3 to 4 days). Long-term cultures suitable for permanent storage or further studies can be derived from a variety of other tissues. Skin biopsy, a minor surgical procedure, can provide samples of tissue that in culture produce **fibroblasts,** which can be used for a variety of biochemical and molecular studies as well as for chromosome and genome analysis. White blood cells can also be transformed in culture to form **lymphoblastoid** cell lines that are potentially immortal. **Bone marrow** has the advantage of containing a high proportion of dividing cells, so that little if any culturing is required; however, it can be obtained only by the relatively invasive procedure of marrow biopsy. Its main use is in the diagnosis of suspected hematological malignancies. **Fetal cells** derived from amniotic fluid (amniocytes) or obtained by chorionic villus biopsy can also be cultured successfully for cytogenetic, genomic, biochemical, or molecular analysis. Chorionic villus cells can also be analyzed directly after biopsy, without the need for culturing. Remarkably, small amounts of **cell-free fetal DNA** are found in the maternal plasma and can be tested by whole-genome sequencing.

Molecular analysis of the genome, including whole-genome sequencing, can be carried out on any appropriate clinical material, provided that good-quality DNA can be obtained. Cells need not be dividing for this purpose, and thus it is possible to study DNA from tissue and tumor samples, for example, as well as from peripheral blood. Which approach is most appropriate for a particular diagnostic or research purpose is a rapidly evolving area as the resolution, sensitivity, and ease of chromosome and genome analysis increase (see Below).

CLINICAL INDICATIONS FOR CHROMOSOME AND GENOME ANALYSIS

Chromosome analysis is indicated as a routine diagnostic procedure for a number of specific conditions encountered in clinical medicine. Some general clinical situations indicate a need for cytogenetic and genome analysis:

- ***Problems of early growth and development.*** Failure to thrive, developmental delay, dysmorphic facies, multiple malformations, short stature, ambiguous genitalia, and intellectual disability are frequent findings in children with chromosome abnormalities. Unless there is a definite nonchromosomal diagnosis, chromosome and genome analysis should be performed for patients presenting with any combination of such problems.
- ***Stillbirth and neonatal death.*** The incidence of chromosome abnormalities is much higher among stillbirths (up to approximately 10%) than among live births (approximately 0.7%). It is also elevated among infants who die in the neonatal period (approximately 10%). Chromosome analysis should be performed for all stillbirths and neonatal deaths that that do not have a clear basis to rule out a chromosome abnormality. In such cases, karyotyping (or other comprehensive ways of scanning the genome) is essential for accurate genetic counseling. These analyses may provide important information for prenatal diagnosis in future pregnancies.
- ***Fertility problems.*** Chromosome studies are indicated for women presenting with amenorrhea and for couples with a history of infertility or recurrent miscarriage. A chromosome abnormality is seen in one or the other parent in 3% to 6% of cases in which there is infertility or two or more miscarriages.
- ***Family history.*** A known or suspected chromosome or genome abnormality in a first-degree relative is an indication for chromosome and genome analysis.
- ***Neoplasia*** **.** Virtually all cancers are associated with one or more chromosome abnormalities. Chromosome and genome evaluation in the tumor itself, or in bone marrow in the case of hematological malignant neoplasms, can offer diagnostic or prognostic information.
- ***Pregnancy.*** There is a higher risk for chromosome abnormality in fetuses conceived by women of increased age, typically defined as older than 35 years. Fetal chromosome and genome analysis should be offered as a routine part of prenatal care in such pregnancies. As a screening approach for the most common chromosome disorders, noninvasive prenatal testing using whole-genome sequencing is now available to pregnant women of all ages.
-

Chromosome Identification

The 24 types of chromosome found in the human genome can be readily identified at the cytological level by specific staining procedures. The most common of these, Giemsa banding (**G banding**), was developed in the early 1970s and was the first widely used whole-genome analytical tool for research and clinical diagnosis. It has been the gold standard for the detection and characterization of structural and numerical genomic abnormalities in clinical diagnostic settings for both constitutional (postnatal or prenatal) and acquired (cancer) disorders.

G-banding and other staining procedures can be used to describe individual chromosomes and their variants or abnormalities, using an internationally accepted system of chromosome

classification. Figure 5-2 is an ideogram of the banding pattern of a set of normal human chromosomes at metaphase, illustrating the alternating pattern of light and dark bands used for chromosome identification. The pattern of bands on each chromosome is numbered on each arm from the centromere to the telomere, as shown in detail in Figure 5-3 for several chromosomes. The identity of any particular band (and thus the DNA sequences and genes within it) can be described precisely and unambiguously by use of this regionally based and hierarchical numbering system.

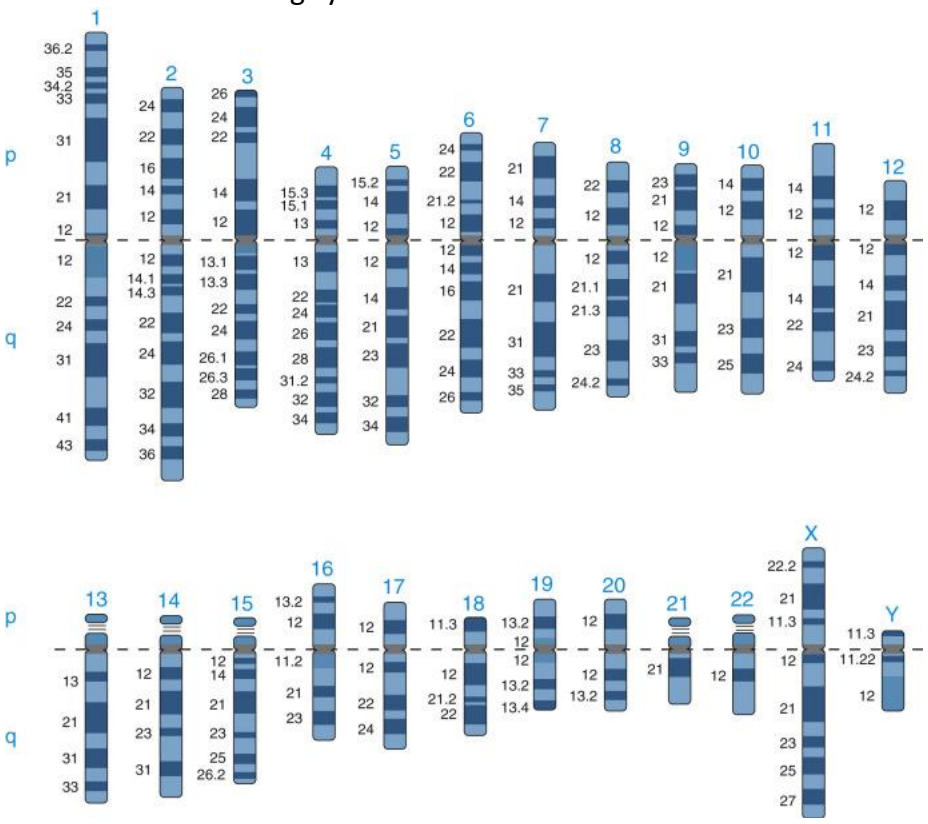


Figure 5-2
Ideogram showing G-banding patterns for human chromosomes at metaphase, with approximately 400 bands per haploid karyotype.
As drawn, chromosomes are typically represented with the sister chromatids so closely aligned that they are not recognized as distinct entities. Centromeres are indicated by the primary constriction and narrow *dark gray* regions separating the p and q arms. For convenience and clarity, only the G-dark bands are numbered. For examples of full numbering scheme, see Figure 5-3 .

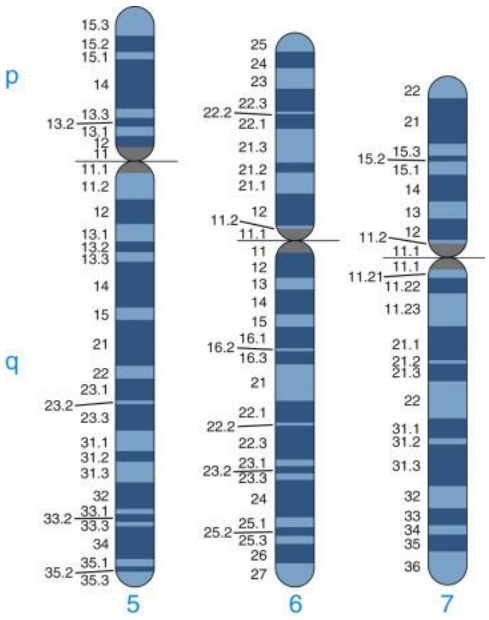


Figure 5-3
Examples of G-banding patterns for chromosomes 5, 6, and 7 at the 550-band stage of condensation.
Band numbers permit unambiguous identification of each G-dark or G-light band, for example, chromosome 5p15.2 or chromosome 7q21.2.

Human chromosomes are often classified into three types that can be easily distinguished at metaphase by the position of the **centromere,** the primary constriction visible at metaphase (see Fig. 5-2): **metacentric** chromosomes, with a more or less central centromere and arms of approximately equal length; **submetacentric** chromosomes, with an off-center centromere and arms of clearly different lengths; and **acrocentric** chromosomes, with the centromere near one end. A potential fourth type of chromosome, **telocentric,** with the centromere at one end and only a single arm, does not occur in the normal human karyotype, but it is occasionally observed in chromosome rearrangements. The human acrocentric chromosomes (chromosomes 13, 14, 15, 21, and 22) have small, distinctive masses of chromatin known as **satellites** attached to their short arms by narrow stalks (called secondary constrictions). The stalks of these five chromosome pairs contain hundreds of copies of genes for ribosomal RNA (as well as a variety of repetitive sequences.

In addition to changes in banding pattern, nonstaining gaps—called **fragile sites** —are occasionally observed at particular sites on several chromosomes that are prone to regional genomic instability. Over 80 common fragile sites are known, many of which are heritable variants. A small proportion of fragile sites are associated with specific clinical disorders; the fragile site most clearly shown to be clinically significant is seen near the end of the long arm of the X chromosome in males with a specific and common form of X-linked intellectual disability, **fragile X syndrome**, as well as in some female carriers of the same genetic defect.

Targeted high-resolution chromosome banding was largely replaced in the early 1990s by **fluorescence in situ hybridization (FISH),** a method for detecting the presence or absence of a particular DNA sequence or for evaluating the number or organization of a chromosome or chromosomal region in situ (literally, "in place") in the cell. This convergence of genomic and cytogenetic approaches—variously termed *molecular cytogenetics, cytogenomics,* or *chromonomics* —dramatically expanded both the scope and precision of chromosome analysis in routine clinical practice.

FISH technology takes advantage of the availability of ordered collections of recombinant DNA clones containing DNA from around the entire genome, generated originally as part of the Human Genome Project. Clones containing specific human DNA sequences can be used as probes to detect the corresponding region of the genome in chromosome preparations or in interphase nuclei for a variety of research and diagnostic purposes, as illustrated in Figure 5-5 :

- DNA probes specific for individual chromosomes, chromosomal regions, or genes can be labeled with different fluorochromes and used to identify particular chromosomal rearrangements or to rapidly diagnose the existence of an abnormal chromosome number in clinical material.
- Repetitive DNA probes allow detection of satellite DNA or other repeated DNA elements localized to specific chromosomal regions. Satellite DNA probes, especially those belonging to the α-satellite family of centromere repeats (see Chapter 2), are widely used for determining the number of copies of a particular chromosome.

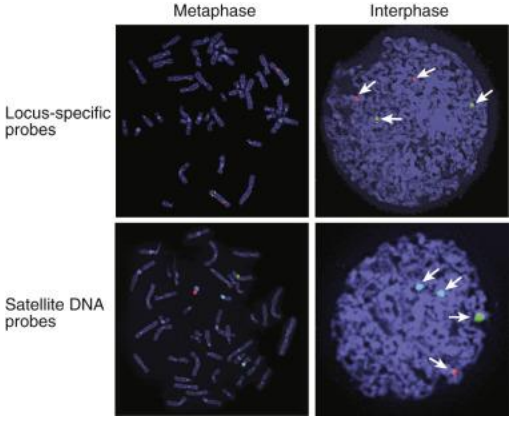


Figure 5-5

Fluorescence in situ hybridization to human chromosomes at metaphase and interphase, with different types of DNA probe.

Top, Single-copy DNA probes specific for sequences within bands 4q12 (*red* fluorescence) and 4q31.1 (*green* fluorescence). *Bottom,* Repetitive α-satellite DNA probes specific for the centromeres of chromosomes 18 *(aqua),* X *(green),* and Y *(red).*

Although FISH technology provides much higher resolution and specificity than G-banded chromosome analysis, it does not allow for efficient analysis of the entire genome, and thus its use is limited by the need to target a specific genomic region based on a clinical diagnosis or suspicion.

Genome Analysis Using Microarrays

Although the G-banded karyotype remains the front-line diagnostic test for most clinical applications, it has been complemented or even replaced by genome-wide approaches for detecting copy number imbalances at higher resolution (see Fig. 5-1), extending the concept of targeted FISH analysis to test the entire genome. Instead of examining cells and chromosomes in situ one probe at a time, chromosomal microarray techniques simultaneously query the whole genome represented as an ordered array of genomic segments on a microscope slide containing overlapping or regularly spaced DNA segments that represent the entire genome. In one approach based on **comparative genome hybridization (CGH),** one detects relative copy number gains and losses in a genome-wide manner by hybridizing two samples—one a control genome and one from a patient—to such microarrays. An excess of sequences from one or the other genome indicates an overrepresentation or underrepresentation of those sequences in the patient genome relative to the control (Fig. 5-6). An alternative approach uses “single nucleotide polymorphism (SNP) arrays” that contain versions of sequences corresponding to the two alleles of various SNPs around the genome. In this case, the relative representation and intensity of alleles in different regions of the genome indicate if a chromosome or chromosomal region is present at the appropriate dosage (see Fig. 5-6).

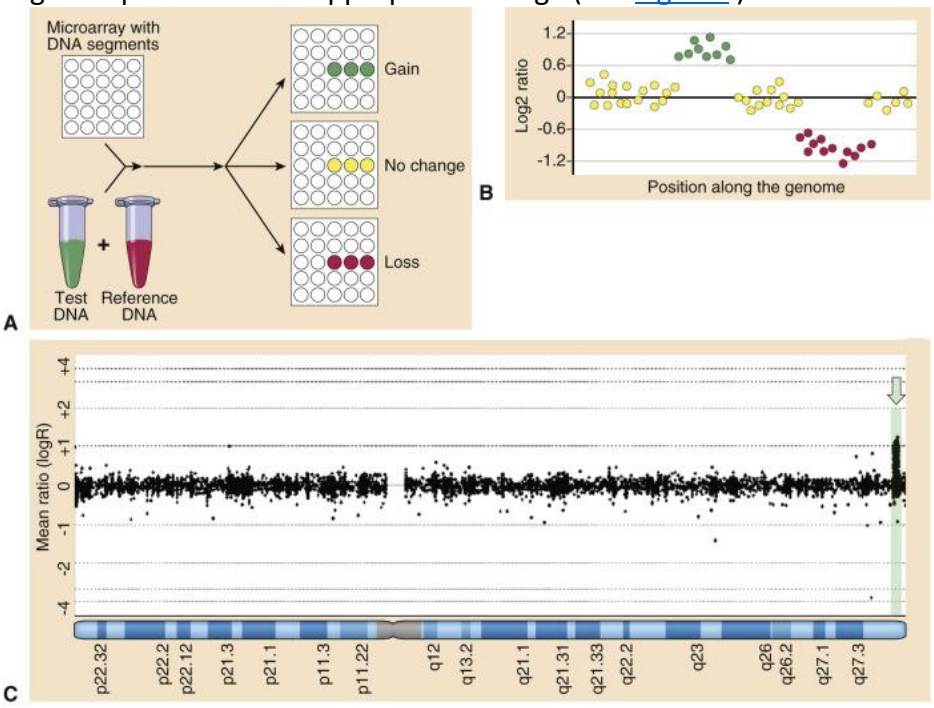


Figure 5-6

Chromosomal microarray to detect chromosome and genomic dosage.

A , Schematic of an array assay based on comparative genome hybridization (CGH), where a patient's genome (denoted in *green*) is cohybridized to the array with a control reference genome (denoted in *red*). The probes are mixed and allowed to hybridize to their complementary sequences on the array. Relative intensities of hybridization of the two probes are measured, indicating equivalent dosage between the two genomes *(yellow)* or a relative gain *(green)* or loss *(red)* in the patient sample. **B** , A typical output plots the logarithm of the fluorescence ratios as a function of the position along the genome. **C** , Array CGH result for a

patient with Rett syndrome, indicating a duplication of approximately 800 kb in band Xq28 containing the *MECP2* gene. LogR of fluorescence ratios are plotted along the length of the X chromosome. Each dot represents the ratio for an individual sequence on the array. Sequences corresponding to the *MECP2* gene and its surrounding region are duplicated in the patient's genome, leading to an increased ratio, indicated by the *green arrow* and *shaded box* in that region of the chromosome.

For routine clinical testing of suspected chromosome disorders, probe spacing on the array provides a resolution as high as 250 kb over the entire unique portion of the human genome. A higher density of probes can be used to achieve even higher resolution (<25-50 kb) over regions of particular clinical interest, such as those associated with known developmental disorders or congenital anomalies (see Fig. 5-6 ;) This approach, which is being used in an increasing number of clinical laboratories, complements conventional karyotyping and provides a much more sensitive, high-resolution assessment of the genome. Microarrays have been used successfully to identify chromosome and genome abnormalities in children with unexplained developmental delay, intellectual disability, or birth defects, revealing a number of pathogenic genomic alterations that were not detectable by conventional G banding. Based on this significantly increased yield, genome-wide arrays are replacing the G-banded karyotype as the routine frontline test for certain patient populations.
Two important limitations of this technology bear mentioning, however. First, array-based methods measure only the relative copy number of DNA sequences but not whether they have been translocated or rearranged from their normal position(s) in the genome. Thus confirmation of suspected chromosome or genome abnormalities by karyotyping or FISH is important to determine the nature of an abnormality and thus its risk for recurrence, either for the individual or for other family members. And second, high-resolution genome analysis can reveal variants, in particular small differences in copy number, that are of uncertain clinical significance. An increasing number of such variants are being documented and catalogued even within the general population. As we saw in Chapter 4 , many are likely to be benign **copy number variants** . Their existence underscores the unique nature of each individual's genome and emphasizes the diagnostic challenge of assessing what is considered a “normal” karyotype and what is likely to be pathogenic.

Genome Analysis by Whole-Genome Sequencing

At the extreme end but on the same spectrum as cytogenetic analysis and microarray analysis, the ultimate resolution for clinical tests to detect chromosomal and genomic disorders would be to sequence patient genomes in their entirety. Indeed, as the efficiency of whole-genome sequencing has increased and its costs have fallen, it is becoming increasingly practical to consider sequencing patient samples in a clinical setting (see Fig. 5-1).
The principles underlying such an approach are straightforward, because the number and composition of any particular segment of an individual's genome will be reflected in the DNA sequences generated from that genome. Although the sequences routinely obtained with today's technology are generally short (approximately 50 to 500 bp) compared to the size of a chromosome or even a single gene, a genome with an abnormally low or high representation of those sequences from a particular chromosome or segment of a chromosome is likely to have a

numerical or structural abnormality of that chromosome. To detect numerical abnormalities of an entire chromosome, it is generally not necessary to sequence a genome to completion; even a limited number of sequences that align to a particular chromosome of interest should reveal whether those sequences are found in the expected number (e.g., equivalent to two copies per diploid genome for an autosome) or whether they are significantly overrepresented or underrepresented (Fig. 5-7). This concept is now being applied to the prenatal diagnosis of fetal chromosome imbalance.

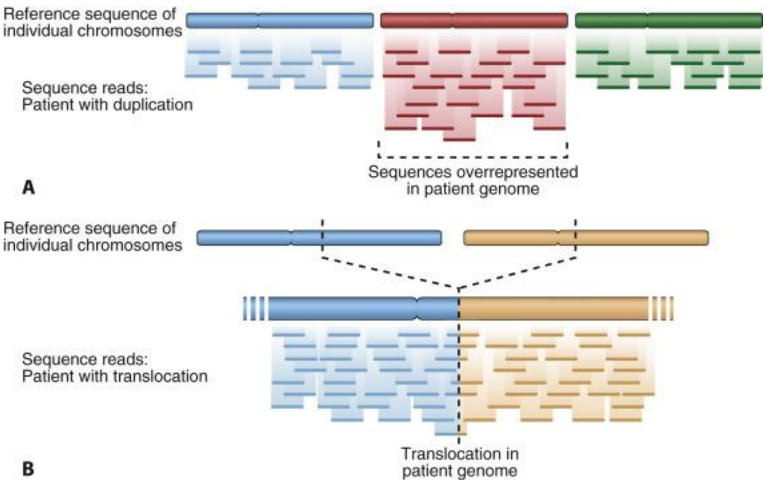


Figure 5-7
Strategies for detection of numerical and structural chromosome abnormalities by whole-genome sequence analysis.
Although only a small number of reads are illustrated schematically here, in practice many millions of sequence reads are analyzed and aligned to the reference genome to obtain statistically significant support for a diagnosis of aneuploidy or a structural chromosome abnormality. **A** , Alignment of sequence reads from a patient's genome to the reference sequence of three individual chromosomes. Overrepresentation of sequences from the *red* chromosome indicates that the patient is aneuploid for this chromosome. **B** , Alignment of sequence reads from a patient's genome to the reference sequence of two chromosomes reveals a number of reads that contain contiguous sequences from *both* chromosomes. This indicates a translocation in the patient's genome involving the *blue* and *orange* chromosomes at the positions designated by the *dotted lines* .
To detect balanced rearrangements of the genome, however, in which no DNA in the genome is either gained or lost, a more complete genome sequence is required. Here, instead of sequences that align perfectly to the reference human genome sequence, one finds rare sequences that align to two *different* and *normally noncontiguous* regions in the reference sequence (whether on the same chromosome or on different chromosomes) (see Fig. 5-7). This approach has been used to identify the specific genes involved in some cancers, and in children with various congenital defects due to translocations, involving the juxtaposition of sequences that are normally located on different chromosomes.

Chromosome Abnormalities

Abnormalities of chromosomes may be either numerical or structural and may involve one or more autosomes, sex chromosomes, or both simultaneously. The overall incidence of chromosome abnormalities is approximately 1 in 154 live births (Fig. 5-8), and their impact is therefore substantial, both in clinical medicine and for society. By far the most common type of clinically significant chromosome abnormality is **aneuploidy,** an abnormal chromosome number due to an extra or missing chromosome. An aneuploid karyotype is always associated with physical or mental abnormalities or both. **Structural abnormalities** (rearrangements involving one or more chromosomes) are also relatively common (see Fig. 5-8). Depending on whether or not a structural rearrangement leads to an imbalance of genomic content, these may or may not have a phenotypic effect. However, as explained later in this chapter, even balanced chromosome abnormalities may be at an increased risk for abnormal offspring in the subsequent generation.

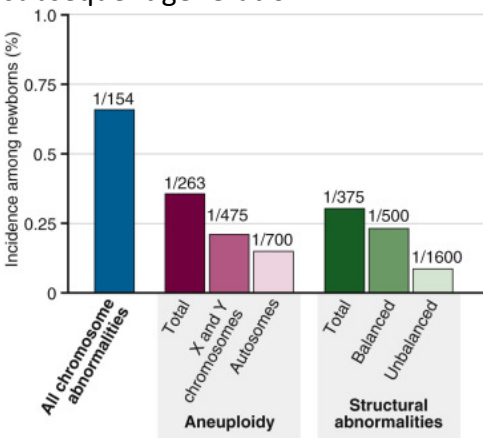


Figure 5-8
Incidence of chromosome abnormalities in newborn surveys, based on chromosome analysis of over 68,000 newborns.

Chromosome abnormalities are described by a standard set of abbreviations and nomenclature that indicate the nature of the abnormality and (in the case of analyses performed by FISH or microarrays) the technology used. Some of the more common abbreviations and examples of abnormal karyotypes and abnormalities are listed in Table 5-1 .

TABLE 5-1
Some Abbreviations Used for Description of Chromosomes and Their Abnormalities, with Representative Examples
Abbreviations from Shaffer LG, McGowan-Jordan J, Schmid M, editors: *ISCN 2013: an international system for human cytogenetic nomenclature* , Basel, 2013, Karger.

Abbreviation	Meaning	Example	Condition
		46,XX	Normal female karyotype
		46,XY	Normal male karyotype
cen	Centromere		
del	Deletion	46,XX,del(5)(q13)	Female with terminal deletion of one chromosome 5 distal to band 5q13
der	Derivative chromosome	der(1)	Translocation chromosome derived from chromosome 1 and containing the centromere of chromosome 1
dic	Dicentric chromosome	dic(X;Y)	Translocation chromosome containing the centromeres of both the X and Y chromosomes
dup	Duplication		
inv	Inversion	inv(3)(p25q21)	Pericentric inversion of chromosome 3
mar	Marker chromosome	47,XX,+mar	Female with an extra, unidentified chromosome
mat	Maternal origin	47,XY,+der(1) mat	Male with an extra der(1) chromosome inherited from his mother
p	Short arm of chromosome		
pat	Paternal origin		
q	Long arm of chromosome		
r	Ring chromosome	46,X,r(X)	Female with ring X chromosome
rob	Robertsonian translocation	rob(14;21)(q10;q10)	Breakage and reunion have occurred at band 14q10 and band 21q10 in the centromeric regions of chromosomes 14 and 21
t	Translocation	46,XX,t(2;8)(q22;p21)	Female with balanced translocation between chromosomes 2 and 8, with breaks in bands 2q22 and 8p21
+	Gain of	47,XX,+21	Female with trisomy 21
−	Loss of	45,XY,−22	Male with monosomy 22

Abbreviation	Meaning	Example	Condition
/	Mosaicism	46,XX/47,XX,+21	Female with two populations of cells, one with a normal karyotype and one with trisomy 21